Development of a Fusarium-wilt-resistant glasshouse tomato variety. ALEXANDER, L. J. The commercial wilt-resistant varieties of tomato now available do not possess sufficient resistance to withstand attacks of Fusarium wilt under Ohio glasshouse conditions. Steam sterilization of the soil gives good results but must be repeated annually. Therefore, it seemed desirable to develop a new variety with sufficient resistance to withstand the disease under Ohio glasshouse conditions. Following publication of the work of Bohn and Tucker, certain of their accessions were artificially inoculated at the fifth or sixth true leaf stage when set out in the growing house. Sufficient resistance was shown to justify the initiation of further breeding work which had as its objective the development of a wilt-resistant variety of the Globe type. Following this trial several crosses and back crosses of the best accessions were made to Globe. The best accessions from these crosses are in the fifth and sixth generations since the last cross to Globe. These have a fair degree of homogeneity. These lines have been selected specifically for resistance to race 1 because it appears to be the most widely distributed. Race 2 is known to be present in only one glasshouse but probably occurs in a few others. Preliminary yield trials are very promising. Wider yield trials are being planned for the spring crop of 1947.

Effect of streptomycin on higher plants. ANDERSON, H. W., and INEZ NIENOW. Streptomycin sulphate was used in a nutrient solution (Shives) to determine effect on seedlings. After germination the seedling roots were immersed in dilutions of from 5 to 200 units/ml. Concentrations beyond 50 units/ml. were toxic to tomato and radish seedlings. Soybeans were not killed but there was marked stunting of lateral roots. Wheat was not injured at 200 units/ml. The characteristic reduction in lateral root development by Streptomycin was similar to the effect of clavacin on corn, bean, and onion roots in earlier studies. Soaking 8 varieties of seeds 12 hours in 200 units/ml. concentration did not prevent germination but when radish was planted in soil, marked injury (stunting and yellowing) was observed. No reduction in damping-off occurred. Assays of plants grown in Streptomycin solution or in sand cultures watered with the solution showed that Streptomycin is absorbed through the roots. Assays indicated that soybean plants grown in 50 units/ml. solution retained 5–10 units/ml. in expressed sap. Plants grown in 100 units/ml. showed higher assays than those grown in 50-unit concentrations. Soybeans containing 4–5 units/ml. when inoculated with *Xanthomonas phaseoli* var. *sojense* had typical symptoms of bacterial pustule, and the organism was readily reisolated from the lesions.

Dutch elm disease recurrence in American elms in relation to the extent of vascular invasion by Ceratostomella ulmi in the year of disease incidence. BANFIELD, W. M. Recurrence of Dutch-elm disease was studied in the season following infection in approximately 700 American elm seedlings that had developed many degrees of vascular invasion as a result of diverse types, locus, and time of infection in the season of initial infection. All trees were in a native stand and varied between 1½ and 4 inches diameter at breast height. The disease did not recur in 276 trees wherein vascular invasion was limited to crown branches. It recurred in 2 of 140 trees wherein vascular invasion was extensive in crown and upper bole. It recurred in 82 of 169 trees wherein vascular invasion was extensive in crown, bole, and roots. Histological examination of these trees revealed vessels of consecutive growth sheaths to be contiguous at numerous points in the root system and in the lower bole. In recovered trees no points were found at which invaded vessels of the sheath of initial infection were in contact with vessels of the new sheath. Invaded vessels of new and old sheaths were contiguous at one or many points in trees in which the disease recurred. Such points of contact were most numerous in the roots, present in the bole, and rare in small branches.

Recurrence of Dutch elm disease in American elms in relation to tree stature. BANFIELD, W. M., E. G. REX, and CURTIS MAY. Wilt and dieback symptoms reappear in the season following infection in American elms as a result of general invasion of the vessels of the new sheath by *Ceratostomella ulmi*. This invasion has its incidence in the infected vessels of the preceding sheath. Trees in which no invasion of new vessels develops from infected vessels of the preceding sheath recover and display no symptoms of disease unless

1

reinfected. Disease recurrence varies with tree stature. The disease recurred in less than 10 per cent of several hundred trees of 1-inch diameter at breast height, which survived the initial year of general vascular invasion. The disease recurred in 49 per cent of 169 trees of 2 to 4.9 inches d.b.h., in 83 per cent of 36 trees of 5 to 9.9 inches, in 92 per cent of 27 trees of 10 to 15 inches, and in all 36 trees, greater than 15 inches d.b.h., on which observations were made. The disease continued to recur each year until death, in all trees but one, wherein it recurred in the second year only. Most trees died in the second season, about a third died in the third season. Three large trees continue as living relicts after 5 years of recurring disease. (Massachusetts State College, N. J. State Department of Agriculture, and U. S. Department of Agriculture.)

Vitamin deficiencies in the Sclerotiniaceae. BARNETT, H. L., and V. G. LILLY. Fifteen species in 8 genera were studied with respect to their growth in the absence and in the presence of 4 common vitamins (thiamin, pyridoxine, inositol, biotin) used separately and in combination. Variations in vitamin requirements were found among the isolates of the same species as well as among different species. Four species (*Sclerotinia sclerotiorum, Botryotinia convoluta, Ciboria pseudotuberosa, Ciborinia erythronii*) were found to be autotrophic with respect to these vitamins. All other species studied were heterotrophic in varying degrees for thiamin or biotin, or both. *Sclerotinia camelliae* and *Lambertella hicoriae* grew well only in a medium containing both thiamin and biotin. Isolates of *Monilinia fructicola, Ciboria acerina,* and *Sclerotinia minor* varied in their response to the vitamins. The growth of certain species was somewhat depressed by pyridoxine or inositol, or both.

Fungicidal mechanism of disodium ethylene bisdithiocarbamate. BARRATT, RAYMOND W., and JAMES G. HORSFALL. Spores of *Macrosporium sarcinaeforme* germinating on glass slides were used to assay the toxicity of numerous derivatives of

$$Na\text{-}S\overset{\overset{\text{S}}{\|}\overset{\text{H}}{|}}{\text{-C-N}}\text{-CH}_2\text{-CH}_2\text{-}\overset{\overset{\text{H}}{|}\overset{\text{S}}{\|}}{\text{N-C}}\text{ S-Na.}$$

The intact molecule may function as previously suggested by precipitating the metals necessary for the enzyme systems of the spore. Lengthening of the aliphatic carbon chain decreased the potency. The theory is advanced that the major part of the toxicity is due to H₂S liberated during decomposition. The equilibrium Na-S-R + HOH ⇌ H-S-R + NaOH existing in solution provides for hydrogen tautomerism on the sulphur $H\text{-S-}\overset{\overset{\text{S}}{\|}\overset{\text{H}}{|}}{\text{C-N}}\text{-R} \rightleftharpoons S\text{=}\overset{\overset{\text{H-S}}{|}\overset{\text{H}}{|}}{\text{C-N}}\text{-R}$, while the hydrogen on the nitrogen provides for tautomerism between nitrogen and sulphur $H\text{-S-}\overset{\overset{\text{S}}{\|}\overset{\text{H}}{|}}{\text{C-N}}\text{-R} \rightleftharpoons H\text{-S-}\overset{\overset{\text{S-H}}{|}}{\text{C}}\text{=N-R.}$

During the dynamic states of the molecule, hydrogen sulphide is evolved: $H\text{-S-}\overset{\overset{\text{S}}{\|}\overset{\text{H}}{|}}{\text{C-N}}\text{-R} \rightleftharpoons$ S=C=N-R (ethylene bis diisothiocyanate) + H₂S. Evidence supporting this view is (1) hydrogen sulphide is evolved in water, (2) conversion of the nitrogen from a secondary to a tertiary amine either by methylation or inclusion in a ring reduces toxicity by the order of 20 fold, presumably by preventing resonance, (3) substitution of the sodium by other soluble ions like potassium and quaternary ammonium has no effect on toxicity, but substitution of insolubilizing metallic ions, such as zinc, strontium, or cadmium, reduces toxicity by the order of 2 fold presumably by reducing the rate of hydrolysis.

Cherry yellows and necrotic ring spot of sour cherry in Ontario. No. 1. The value of Prunus persica and P. domestica var. Italian prune as index hosts. BERKELEY, G. H. Necrotic ring-spot symptoms, consisting of delayed foliation, chlorotic spots, rings, and necrosis of leaves, are of the shock type, since affected trees recover, while symptoms of cherry yellows, which consist of yellowing of leaves, which are cast, are of the chronic type in that they recur year after year. Budding experiments indicated that every source of cherry yellows tested has also contained the necrotic ring-spot virus. But necrotic ring spot has been found to occur free from the yellows virus. These experiments indicated that peach seedlings, or the varieties Elberta or Rochester, are of value as indicator hosts. Peaches reacted to necrotic ring-spot virus by causing shock symptoms of delayed foliation, die-back, and bark necrosis, followed by recovery provided the initial shock effect was not too severe. Peaches reacted to the cherry-yellows complex by producing shock symptoms similar to those caused by the necrotic ring-spot virus and a chronic symptom of rosetted shoots which persist. Italian prune reacted somewhat similarly by causing a shock symptom of leaf necrosis with either necrotic ring-spot virus alone, or with the cherry-yellows complex, while the latter in

addition produced chronic symptoms similar to those associated with prune dwarf. However, the experimental evidence does not indicate that cherry yellows and prune dwarf are caused by the same virus or virus complex. Variation in degree of symptoms resulting from inoculation suggests the presence of strains of both viruses.

Alfalfa mosaic on pepper in Ontario. BERKELEY, G. H. An unusual type of mosaic was found on sweet pepper var. California Wonder in Ontario in 1943 and again in 1944. Symptoms included chlorotic rings, spots, and patterns on both leaves and fruit. Preliminary inoculations indicated that the causal virus was closely related to *Marmor medicaginis* II. A large number of inoculations, extending over a 3-year period, using *Marmor medicaginis* H. var. *typicum*, *M. medicaginis* II. var. *solani*, and the pepper virus indicated that the pepper virus is a strain of *Marmor medicaginis*. Comparative studies were made on *Nicotiana tabacum*; *N. rustica*; *N. glutinosa*; *Vicia faba*, broad bean; *Lathyrus odoratus*, sweet pea; *Pisum sativum*, pea; *Phaseolus vulgaris*, bean; *Trifolium pratense*, red clover; *Capsicum frutescens*, pepper; *Soja max*, soybean; *Solanum melongena*, eggplant; *Zinnia elegens*, zinnia; *Cucumis sativus*, cucumber; *Petunia hybrida*. petunia; *Lycopersicon esculentum*, tomato; *Antirrhinum majus*, snapdragon; and *Apium graveolens*, celery. With the following exceptions, all 3 viruses produced similar symptoms on the above hosts. The pepper virus did not infect tomato, while both the alfalfa-mosaic virus and the potato-calico strain did. Whereas the pepper virus and the alfalfa-mosaic virus gave positive results on cucumber, negative results were obtained with the potato-calico virus. The potato-calico strain produced prominent calico symptoms with wide vein-banding on *N. rustica* and *N. glutinosa*. On these same hosts alfalfa-mosaic virus and the pepper virus produced much less prominent symptoms with the wide vein-banding lacking. The pepper virus produced more severe necrosis on *N. glutinosa*, *N. rustica*, *N. tabacum* than did alfalfa virus I or the potato-calico virus. Cross protection and thermal death point tests also indicated that the pepper virus was a strain of *Marmor medicaginis* H.

A virus disease of the potato transmitted by the aster leafhopper. BONDE, REINER, and E. S. SCHULTZ. The aster leafhopper, *Macrosteles divisus*, transmitted by artificial inoculations a virus disease of the potato which appears to be different from purple-top wilt. The symptoms of the disease are apparent toward the end of the growing-season and are characterized by a slight dwarfing and by a rolling of the upper leaves of the plant similar to that often associated with rhizoctonia stem rot and other diseases. The stems of the pigmented varieties develop a purplish color. The internodes may become shortened, and the nodes enlarged. The plants do not wilt or die prematurely, and a pronounced necrosis of the vascular bundles near or at the ground line does not occur. None of the tubers from diseased plants becomes flabby or develops vascular discoloration. They germinate normally and the disease is perpetuated through the seed tubers. This disease has been found in potato fields in Aroostook County, Maine, but apparently is of little economic importance. The disease is very similar to apical leafroll, previously described by Schultz and Bonde. (Maine Agricultural Experiment Station and United States Department of Agriculture.)

Necrosis of soybean stem and root caused by Rhizoctonia solani. BOOSALIS, M. G. On stems and roots of soybean attacked by *Rhizoctonia solani* are necrotic lesions in which a browning and discoloration of host cells may extend for 5 to 8 cell layers in advance of the fungus hyphae. Hyphae were abundant on the epidermis, but never penetrated the host without first discoloring the host cells. Staling products were filtered from cultures of *Rhizoctonia*. Soybean seeds were germinated in the extracts, or the stems of aseptically grown soybean seedlings were wrapped with narrow strips of cotton soaked with the extract. Staling products alone, the diffusible substances produced by the fungus, reduced germination of soybean seed, inhibited development of secondary roots, caused necrotic lesions on stems and roots of seedlings, and killed seedlings within 7 to 10 days. Necrotic lesions produced by the extracts resembled lesions caused by hyphae of *Rhizoctonia*, but *Rhizoctonia* could not be isolated from them. No necrotic lesions appeared on control plants. (Minnesota Agricultural Experiment Station and United States Department of Agriculture.)

A three-spray schedule for the control of black rot of grapes. BRAUN, ALVIN J. Tests conducted in New York State vineyards indicate that under most conditions, black rot, *Guignardia bidwellii* (Ell.) Viala and Ravaz, can be effectively controlled by 3 applications, immediately before bloom, immediately after bloom, and 10–14 days later. Under conditions of severe disease occurrence, the percentage of control obtained with the various materials tested was significantly less, indicating the necessity of an additional pre-bloom application in vineyards where the disease is likely to be severe. Two late-season applications did not increase the control in those plots that already had

received 3 applications. Fermate (ferric dimethyl dithiocarbamate) 2–100 was more effective than any of the other fungicides tested, including Bordeaux 8–8–100. Bordeaux mixture caused injury to the foliage characterized by premature yellowing, especially in those plots receiving 5 applications of the 8-8–100 concentration. The foliage in the plots sprayed with Fermate retained a dark green healthy appearance, even after the foliage of the check plots showed signs of advanced maturity.

Correlated inheritance of resistance to eight races of wheat leaf rust, Puccinia rubigovera tritici, powdery mildew, Erysiphe graminis tritici, and glume color. CALDWELL, R. M., and L. E. COMPTON. The inheritance of seedling resistance to leaf rust races 9, 31, 65, 78, 79, 80, 101, and 110 and to two cultures of powdery mildew was studied by means of greenhouse inoculations, in F_3 and bulk F, progenies of individual F_2 plants of the cross Wabash C.I. 11384 × Michigan Amber 29–1–1–1, C.I. 4770. Rust reactions were determined on the first and mildew reactions on the third and fourth leaves of seedlings. The first leaf of Wabash gives either the resistant or "X" reaction to each of the 8 races. The "X" reaction usually changes to a resistant type in the second or third leaves. Michigan Amber was resistant to the two cultures of powdery mildew studied. Wabash has white glumes and Michigan Amber, brown. The segregation indicated a monogenic inheritance of resistance to leaf rust. Susceptibility was dominant. Each progeny reacted uniformly to the group of 8 races indicating that for all 8 races the same gene controlled either the resistant or "X" reaction. Mildew resistance and brown chaff color appeared to be monogenic and dominant. Leaf rust and mildew resistance and chaff color were independently inherited, the deviation from a phenotypic ratio for 3 factors being insignificant. (Purdue Agricultural Experiment Station and U. S. Department of Agriculture.)

Inheritance of resistance to loose smut of wheat, Ustilago tritici, in the varietal cross Trumbull × Wabash. CALDWELL, R. M., and L. E. COMPTON. During the 1939 and 1940 field seasons, respectively, 101 and 98 F_2 plants of the cross, Wabash × Trumbull, were inoculated by the partial vacuum technique with a culture of loose smut to which Trumbull is resistant and Wabash susceptible. The F_3 progenies of the inoculated plants were grown in the field the years following inoculation when the percentages of heads infected were recorded. In both years approximately 75 per cent of the F_3 families fell in the range of 0 to 5 infection with the remainder distributed over the approximate range 5 to 85 per cent infected heads. F_2 populations of the same cross grown from seed from inoculated F_1 plants have produced only occasionally infected plants. Reciprocally crossed seed of the parentage, Wabash × Trumbull, containing the F_1 embryos have produced smut-free F_1 plants with few exceptions when inoculated by hand with a smut suspension. Backcrossed seed of the parentage Wabash ♀ × (Wabash × Trumbull) ♂ and inoculated with smut suspensions have produced partially infected progenies. These results suggest that Trumbull's resistance is dominant and monogenically inherited and that infection of the susceptible progeny of heterozygous resistant plants is prevented by the covering parental tissues of the ovary integuments or nucellus. (Purdue Agricultural Experiment Station and U. S. Department of Agriculture.)

Studies on the nature of "segregation" in certain plus strains of Glomerella. CHILTON, S. J. P., and H. E. WHEELER. Ascospores isolated from certain perithecial and nonconidial strains of *Glomerella* known as plus produced a high percentage of new strains known as minus. Occasionally, conidial but nonperithecial strains were found in the ascospore progenies. Analyses of asci gave ratios of 8 plus, 4 plus and 4 minus, or 8 minus cultures. The percentages were 8, 60, and 32, respectively. Only one ascus ratio occurred in a perithecium. Appropriate crosses among these strains indicated the new strains to be genetically different from the plus parental strain, the minus differing at one locus and the conidial at another. The two loci were apparently loosely linked. Ascospores of the plus strain were uninucleate. The evidence indicates that the new strains arose by mutation from the plus strain and the ascus ratios were determined by the nuclei entering and pairing in the ascogenous hyphae and fusing in the ascus.

Transfer of wildfire resistance from Nicotiana longiflora to N. tabacum. CLAYTON, E. E. A selection of *Nicotiana longiflora* was immune from the wildfire (*Phytomonas tabaci*) disease, while cultivated tobacco was susceptible. After prolonged efforts a cross was successfully made in 1940. Tetraploidy was not involved and progeny of the first backcross to tobacco (N.t. × N.l. × N.t.) were self- and cross-fertile. Inoculation and selection work produced an F_6 in 1944 that appeared to possess *N. longiflora* immunity from wildfire. This genotype was designated as T.I. 106. Tests were conducted with T.I. 106 in 1945. Inoculations with *P. tabaci* were begun with seedlings when they appeared above ground in the plant bed and continued until the end of the growing season in the field. No infection was secured. The tests were repeated in 1946 with the same

results. Adjacent checks of common tobacco were 100 per cent severely affected. T.I. 106 was crossed with varieties of commercial tobacco and 5 F_1's were planted in 1946. The F_1's reacted the same as the T.I. 106 parent, hence resistance was dominant. All F_1's showed marked heterosis. Increases in green weight ranged from 25 to 51 per cent, the comparisons being made with the commercial tobacco parents, which were larger than T.I. 106. Inoculation tests with *P. angulata* indicated that T.I. 106 was resistant to blackfire as well as wildfire.

The "dodder graft," a new method of using dodder to transmit plant viruses. COCHRAN, G. W. The "dodder graft" was devised to improve the dodder method of transmitting viruses. Stocks were rooted healthy plants, while scions were detached diseased stems or leaves. Stems of stock and scion were usually placed together and connected by detached dodder tips wound in a circular or figure-eight manner. Winding was facilitated by using a paste of Carbowax 4000 with 15 per cent water. An inch or more of the stems were wound and tied with string. The Carbowax was then removed with water. The "dodder grafts" were sprayed with a mixture of Fermate, Omilite, and AO_3 to prevent a rotting caused by an unidentified *Botrytis sp.* Then they were continuously atomized with water under incandescent lights for 10 to 14 days. The dodder usually made haustorial connections to both stock and scion in 3 to 4 days. The stocks were shaded on the fifth day and sprayed with 100 p.p.m. of indole butyric acid on the seventh day. The scion and connecting dodder were removed on the tenth to fourteenth day. When the "dodder graft" method is used, virus transmission is improved because of the increased number of shortened dodder stems connecting the diseased and healthy tissues.

Injury to root tissue caused by plant residues. COCHRANE, VINCENT W. The decomposition products of plant residues have been described as directly or indirectly injurious to plant roots. Direct injury to root tissue can be assayed by exposing the radicle of 72-hour-old radish seedlings to the action of cold-water extracts of the ground plant tissue. Extracts made from undecomposed ladino clover and from rye grass caused browning of radish roots in 8 hours. Decomposition of these residues at 2 moisture levels resulted in a progressive disappearance of the factor causing the injury, although microbial action brought about temporary increases in toxicity. Decomposition of ladino clover tissue at 56° C. accelerated destruction of the toxic factor, presumably as a result of the activity of thermophilic bacteria. Apart from this case, studies of microbial populations in the decomposing residues failed to establish any significant association of particular groups of microorganisms with the appearance or disappearance of root injury. Extracts from nondecomposed corn stover did not cause root browning; microbial action under anaerobic conditions caused a transitory appearance of injurious activity. Extracts of soybean tissue did not cause discoloration of radish roots, whether tested before, during, or after decomposition.

A method of forecasting late-blight epiphytotics in Eastern Virginia. COOK, HAROLD T. Late-blight epiphytotics have occurred on early potatoes in Eastern Virginia in only 2 of the last 17 years (1930–46) and on tomatoes in but one year. Routine use of fungicides has not been justified because of the infrequent occurrence of blight, but spraying or dusting would be valuable in blight years if epiphytotics could be predicted. Analysis of meteorological data for the 17 years show that epiphytotics may be predicted by plotting the average weekly temperature and cumulative rainfall for May and June on ordinary cross-section paper, and that the advisability of spraying may be determined by this means at weekly intervals during the growing-season. These data show that the development of epiphytotics is dependent upon a combination of above normal rainfall and temperatures below 75° F. from the middle of May to at least the middle of June. Such a combination occurred only in 1938 and 1946, when blight was prevalent. Either rainfall or temperature, or both, were unfavorable for blight in the other 15 years. The tomato crop was damaged only in 1946 when temperatures below 75° F. continued through June.

Occurrence of Actinomyces antibiotic to Pythium in some sugar-cane soils of Louisiana. COOPER, V. E., and S. J. P. CHILTON. In studies initiated in the sugar-cane area of Louisiana on the Pythium root rot of sugar cane, a survey was made in 1946 of the antibiotic *Actinomyces* present in the soils of the cane area. Of 3788 cultures tested against a parasitic strain of *Pythium* and classified according to the distance at which the *Pythium* was inhibited in Petri-dish tests, 896, or 23.6 per cent, showed some antibiosis. Of these 365 inhibited *Pythiums* at 1 to 5 mm.; 301 inhibited at 6 to 10 mm.; 140 at 11 to 15 mm.; 90 at 16 or more mm.; and only 10 inhibited at 35 or more mm. A progressive increase of the Actinomyces populations occurred from March to August, but the percentage of antibiotic strains in the samples tested decreased. The percentage

of antibiotic strains and the average antibiosis of the strains was lower in heavy or root-rot soils than in the light soils.

A bacterial storage disease of sweet potatoes. COX, C. E., and D. W. SQUIRES. An apparently undescribed disease of Maryland Golden sweet potatoes was observed in an experimental storage house on the University of Maryland farm in 1945. A possibly identical storage rot has been reported from a commercial storage house elsewhere in the State. The disease typically involved the entire root, which, externally, became chocolate-brown in contrast with the orange color of healthy sweet potatoes. Diseased potatoes were flaccid and flexible but not mushy. Internally, they were grayish-brown and water-soaked, but the cells did not separate as in soft rot. The odor was distinctive and acrid rather than putrescent. Unless attacked by secondary invaders, the affected roots even-tually dried out and shriveled to form hard mummies. A bacterium has been isolated from diseased tissues. This organism is capable of reproducing symptoms of the disease in sweet potato slices inoculated in the laboratory. It did not cause decay of slices of white potato, tomato, onion, or carrot inoculated similarly. The organism is a short rod, truncate at the ends, nonmotile and Gram-negative. It grows slowly and sparsely on potato-dextrose and malt-extract agars, but makes rapid and fairly abundant growth on sweet-potato-decoction agar.

Specificity of certain organic nitrogen compounds for bacterial plant pathogens. DAVIDSON, RICHARD S. The bactericidal activity of four chemical groups comprising 15 quaternary nitrogen compounds was determined for the following bacteria associated with plant diseases: *Aerobacillus polymyxa*, *Bacillus mesentericus*, *Erwinia carotovora*, *Phytomonas michiganensis*, *Corynebacterium sepedonicum*, and *Bacterium solanacearum*. The groups, in the order in which they produced 100 per cent kill *in vitro* of 24-hour broth cultures, after 10 minutes exposure, were quinoliniums, pyridiniums, nicotiniums, and quaternary ammoniums. Lauryl isoquinolinium bromide was the most active of the group as a bactericide. The least potent of the group was di-methyl di-lauryl ammonium thiocyanate. *A. polymyxa* and *B. mesentericus*, both spore-formers, were killed by higher dilutions of the compounds included in the test than were the nonspore-formers. Of the nonspore-formers included in this study, the Gram-positive bacteria, *P. michiganensis* and *C. sepedonicum*, were killed by higher dilutions of the compounds studied than were the Gram-negative bacteria, *E. carotovora* and *B. solanacearum*.

Control of late blight of tomato in New Jersey. DAVIS, B. H., and C. M. HAENSELER. Late blight, caused by *Phytophthora infestans*, became prevalent in 1946 on tomato foliage in canhouse areas the first week in July. Almost continuous spread followed, the peak of fruit destruction occurring August 20 to 25. Spraying experiments were started in a commercial field 3 days after scattered foliage infection was noted. Eight applications were made at 10-day intervals using a 5-row power take-off sprayer with 3 nozzles per row. Microgel, a tribasic copper sulphate containing 50 per cent copper (4–100), Fermate (ferric dimethyldithiocarbamate) (2–100) alternating with Microgel (4–100), and Zerlate (zinc dimethyldithiocarbamate) (2–100) each containing calcium arsenate (4–100) were used. Control plots received calcium arsenate and lime (4-8–100). Yield and disease data were taken on 100 plants in each treatment. At weekly pickings all ripe cull fruits and rotted green fruits were picked, counted, and discarded. All remaining ripe fruits were then picked, weighed, counted, and marketed. Calculated yields in tons of marketed fruits per acre were: Microgel, 27.7; alternating sprays, 21.4; Zerlate, 13.8; control, 10.2. Of these marketed fruits, 0.2, 2.0, 13.3, and 10.8 per cent were slightly infected. Of the total fruits produced 0.7, 12.1, 55.9, and 59.8 per cent were infected.

Some factors influencing inoculation of tobacco with streak virus. DIACHUN, STEPHEN. Leaves slightly below the primary growing point of systemically infected plants of *Nicotiana rustica* were a more reliable source of tobacco-streak virus than tobacco or sweetclover tissue. Local lesions developed on every one of several hundred tobacco leaves dusted with 600-mesh carborundum and rubbed with a glass spatula dipped in *N. rustica* tissue crushed with M/10 Na$_2$HPO$_4$. Without the M/10 Na$_2$HPO$_4$ and car-borundum the number of local lesions was greatly reduced. Dilution of the inoculum with M/10 Na$_2$HPO$_4$ beyond 1:2 decreased the number of local lesions, but the inoculum was still effective at a dilution of 1:32. Additional rubbing, up to a certain point, increased the number of local lesions. Leaves supported on a flat surface during rubbing did not develop more lesions than leaves merely held at the tip. Very young and old leaves were not so susceptible as vigorous medium-young leaves (5th or 6th below growing point), especially on relatively small plants.

Reaction of 35 species of Nicotiana to tobacco-streak virus. DIACHUN, STEPHEN, and W. D. VALLEAU. None of the approximately 200 accessions of tobacco tested seemed sufficiently resistant to the tobacco-streak virus to be useful as breeding stock. Thirty-five of the approximately 60 described species of *Nicotiana* have been inoculated. The reaction ranged from a high degree of resistance to extreme susceptibility. Most of the species were moderately susceptible, similar to *N. tabacum*. In *N. bethamiana, N. cleve-landii, N. megalosiphon,* and *N. miersii* systemic infection caused severe necrosis, often resulting in death. *N. glauca* and *N. gossii* seemed sufficiently resistant to have value as breeding material.

Symptoms of Dutch elm disease reproduced by toxins of Graphium ulmi in culture. DIMOND, ALBERT E. *Graphium ulmi,* grown on synthetic media, either in shake or quiet submerged culture, produces at least 2 metabolites toxic to elm and tomato cuttings. Each toxin produces symptoms typical of a part of the syndrome of Dutch elm disease. These toxins, produced in 1 month in quiet culture, are as abundantly produced in 4 days in shake culture. The raw culture is filtered through paper, evaporated *in vacuo,* and centrifuged to remove mycelial fragments. The highly toxic clear super-natant liquid in 70 per cent alcohol precipitates a gummy amorphous compound. This is a polysaccharide which gives the red color of a dextrin with IKI, and is hydrolyzed to reducing sugar in hot 1 per cent H_2SO_4. On tomato and elm cuttings it produces an upcurling of leaflets and leaves with marginal withering of the blade, but causes no necrosis. The second fraction is soluble in 70 per cent alcohol, producing a striking interveinal necrosis on tomato and elm. Ether extraction of this fraction to remove organic acids does not alter its toxicity. The ether insoluble, alcohol- and water soluble fraction produces severe interveinal necrosis on tomato cuttings.

Culture techniques for large numbers of diseased and dead wood specimens. FATE, LESTON R., CLYDE E. DIKE, and O. N. LIMING. Standard techniques have been modified and new ones developed for handling several hundred specimens daily in the Dutch Elm Disease Identification Laboratory. Specimens from diseased but living parts of elm trees infected with *Ceratostomella ulmi* or other vascular parasites are cultured on a simplified potato sucrose agar medium. The cultures are held for 3 days at 50° F. and then for 3 days at room temperature. Dead wood and bark materials are cultured by a wet-plate technique, employing excess water and low temperature incubation for 21 days and then room temperature for three days. Low temperature suppresses the growth of most other organisms, while *C. ulmi* is developing. The warm-up period, during which the coremia mature, is an important step in the technique. Through the use of anti-septic solutions, the upward direction of air currents, and low-temperature incubation, laboratory contaminations are satisfactorily controlled.

Identification of Ceratostomella ulmi in pure cultures and in mixed associations of organisms. FENNER, LAWRENCE M., and O. N. LIMING. Zonate colony growth and conidiophores with young spores form the basis for identification of *Ceratostomella ulmi* on potato-sucrose agar. Coremia may form on the wood plantings, but usually not on the agar medium. *Verticillium* and *Cephalosporium* isolations form different type colonies. In wet plate cultures, *C. ulmi* coremia have translucent white heads which may droop or sink over the stalk, and develop surface striations when exposed to dry air. Six other *Graphium* species have different stalk and head characteristics. Several sapro-phytes and air contaminants may over-run and mask *C. ulmi* mycelium and coremia. *Dothiorella ulmi* and a *Cytospora* limit *C. ulmi,* whereas *Sphaeropsis ulmi* and a *Dematium* favor *C. ulmi* coremial formation.

Pathogenicity of isolates of the onion pink-root organism. GORENZ, A. M. Various isolates of *Phoma terrestris* were tested in sand culture on Yellow Globe, a very sus-ceptible variety, and Yellow Bermuda, a moderately resistant one. Severe damping-off along with the typical discoloration of the roots occurred on Yellow Globe seedlings with the 2 most virulent isolates, 1 from Texas and 1 from Louisiana. On Yellow Bermuda, damping-off was less common, but typical discoloration of the roots was present. Iso-lates from Utah, Colorado, Iowa, and Wisconsin were less virulent and caused only typi-cal root discoloration. Pycnidial production on corn-meal agar was observed in the Louisiana, Texas, Iowa, and Colorado isolates. In plants infected with the Louisiana isolate mature pycnidia were produced abundantly on the roots, this being the first record of the development of pycnidia on the host. (University of Wisconsin and U. S. Depart-ment of Agriculture.)

Influence of methocel sticker on the effectiveness of Arasan for onion-smut control. GORENZ, A. M., and J. C. WALKER. Onion-seed-treatment experiments for the control of smut (*Urocystis cepulae*) were conducted in southeastern Wisconsin in 1946. Arasan

(tetramethyl-thiuram-disulphide) was used with and without methocel sticker. Seed was sown at approximately 65 lb. per acre, the usual rate for production of onion sets. In one series of experimental plots an average of 49.8 per cent of the plants from seed with no treatment were diseased. When $\frac{1}{16}$ lb. of Arasan per pound of seed was applied without sticker the percentage of diseased plants was 3.8. When $\frac{1}{8}$ lb. was applied with sticker there was a significant increase in disease to 9.8 per cent. When the dosage was increased to $\frac{1}{4}$ lb. with sticker there was a significant decrease to 2.5 per cent. In another series of trials on a commercial scale there was a similar increase of disease when $\frac{1}{8}$ lb. was applied with sticker over $\frac{1}{16}$ lb. without sticker. In commercial practice results with $\frac{1}{16}$ lb. without sticker were variable, while with sticker more uniform results were secured but a dosage of $\frac{1}{4}$ lb. was necessary for maximum control. (University of Wisconsin and U. S. Department of Agriculture.)

Growth of Streptomyces griseus in shake-flasks. GOTTLIEB, DAVID, and H. W. ANDERSON. The growth of *Streptomyces griseus* was studied by 3 methods, (1) microscopic observation, (2) mycelium weight, and (3) viscosity measurement. Exact time of spore germination was difficult to determine, since, even under oil immersion, only an elongation of the spores was observed. At 6 hours the mycelium was sparse and consisted of some small individual hyphae and of longer branched hyphae which tended to round up into a loose weft. With increased growth, the weft formed masses of mycelium consisting of a dense solid center and a periphery of branched radiating hyphae. At 24 to 30 hours the entire body of medium was filled with these clumps. The peripheral hyphae from the central cores intermingled and produced a continuous mass of intertwining hyphae. The culture appeared very viscous at this stage. After 48 hours the mycelium began to fragment and spores were produced. The number of spores increased, and at 84 hours a lysis of the mycelium was apparent. The dense central core also disintegrated into granular pieces. The culture became less viscous during this stage. Both viscosity and mycelial weight determination showed an increase, to a maximum at 24–30 hours, then a decrease up to about 96 hours followed by a gradual levelling.

Investigations on grey speck of oats in Manitoba. HAGBORG, W. A. F. A disease, thought to be grey speck, was found in varietal plots at Winnipeg and in farmers' fields at several other localities in Manitoba. Pot experiments in 1944 furnished proof that the disease was grey speck and in subsequent field experiments statistically significant increases in yield were obtained following the use of manganese sulphate. When manganese sulphate was applied to the soil (65 lb. MnSO$_4$/acre) the increase in yield was 58 bu./acre; when applied as a spray (9 lb. MnSO$_4$/acre) it was 24 bu./acre; when applied as a 25 per cent dust diluted with clay (8 lb. MnSO$_4$/acre) it was 20 bu./acre; and when applied as a seed steep (9.5 lb. MnSO$_4$/acre) it was 12–17 bu./acre. Marked differences were observed in varietal susceptibility to grey speck. In a replicated test of 68 varieties in 1946, *Avena strigosa*, Black Mesdag, Ajax, Exeter, and Laurel were among the more resistant varieties, while Victoria, Tama, Vicland, Bonda, Early Miller, Valor, Sixty-Day, Erban, Trispernia, Mindo, Legacy, and Bond, were among the more susceptible. High susceptibility has been noted among varieties descended from Victoria, but there is not a complete linkage between resistance to stem and crown rusts and susceptibility to grey speck.

A plot inoculation method for determining the resistance of wheat varieties to bacterial black chaff. HAGBORG, W. A. F., and R. F. PETERSON. A simple method of inoculation for initiating infection with bacterial black chaff in field plots was found effective in a yield test of 98 varieties of wheat. After spraying the plots with an aqueous suspension of *Xanthomonas translucens* f. sp. *undulosa* (S.J. & R.) Hagb., a cloth, moistened with the suspension, was dragged over the plants. Three of the 6 replicates were inoculated and 3 left noninoculated. Comparable means for inoculated and noninoculated plots, respectively, were as follows: leaf infection, per cent, 39.7, 5.1; head infection, per cent, 18.7, 8.4; yield, bu./acre, 39.4, 43.3. The 3 differences were statistically significant (P < 0.05). Leaf and head infections were significantly correlated: varietal means r = 0.52 (P < 0.01), plots within varieties r = 0.38 (P < 0.01). Analyses of variance of the data from the three inoculated replicates, with the varieties considered in 10 groups based on ancestry, established the following significant differences between varietal means: leaf infection, all varieties P < 0.01; leaf infection, varieties within each of 9 groups P < 0.05; head infection, all varieties P < 0.01; head infection, varieties within each of 4 groups P < 0.05. Differences between group means were highly significant: leaf infection P < 0.01, head infection P < 0.01. A sound basis existed, therefore, for selecting varieties resistant to bacterial black chaff. (University of Manitoba and Dominion Laboratory of Cereal Breeding.)

A new disease of garden pea. HARE, WOODROW W. A species of *Pythium* was isolated from dead tips of garden pea (*Pisum sativum*) in 1941 and its pathogenicity to

young foliage of peas determined. This disease caused 5–10 per cent loss in experimental plantings in 1946, and isolations gave the same *Pythium*. In the field, infection occurs in the axils of young leaves or in the bud of the plant. A single leaf may be killed but usually the entire tip is killed by infection in the bud or from the leaf axil. Affected tissues collapse quickly and become dark-brown. Only the upper 3 to 5 internodes are killed in most cases and new branches are put out below. Greenhouse inoculations of potted plants with mycelial-fragment suspensions in buds or leaf axils gave similar symptoms. The disease has been seen only after periods of heavy rain.

Heptadecylglyoxalidine (341) and its hydrolysis product as fungicides. HARRY, JOHN B., R. W. McNAMEE, and R. H. WELLMAN. In 1946, 2-heptadecylglyoxalidine was melted and slurried in hot water to provide a dispersible paste. In previous years it had been formulated as a hydrochloride mixed with pyrophyllite, or as a cold, wet, ball-milled product. The 1946 slurry failed to control apple scab and controlled cherry leaf spot less effectively than did previous products. It was found that under the conditions of preparation of the slurry, the glyoxalidine had hydrolyzed. The hydrolysis product is less effective than the parent glyoxalidine in laboratory spore-germination tests and greenhouse disease-control tests. It can be distinguished from the glyoxalidine by spectral analysis or by its biological effectiveness. The wet ball-milled slurry hydrolyzes much more slowly than the hot-mixed slurry produced in 1946. In the time lapse between wet ball-milling and field application in 1942, the glyoxalidine was only partly hydrolyzed, and good control of apple scab was obtained. The hydrochloride was not hydrolyzed at all. Past results have been best when the hydrochloride was used. (Crop Protection Institute and Carbide and Carbon Chemicals Corporation.)

Zinc ethylene bisdithiocarbamate as a fungicide on vegetables. HEUBERGER, J. W., S. H. DAVIS, JR., L. P. NICHOLS, and L. D. BUEHLER. In 1946, zinc ethylene bisdithiocarbamate (reaction product, coded He 178c) was tested as a spray at the rate of 1½–100 active ingredient on potatoes, tomatoes, cucumbers, cantaloupes, and celery to evaluate its disease controlling powers, phytotoxicity, and effect on yield. *Disease control:* Of all the copper and organic fungicides tested (Bordeaux, Compound A, Tribasic, Yellow Cuprocide, Zerlate, Dithane + zinc sulphate-lime, CPI 169A, CPI 341) zinc ethylene bisdithiocarbamate gave the best control of early and late blight diseases on potato and tomato, of anthracnose fruit spot on tomato, of cucumber downy mildew, and of Cercospora leaf spot on celery. On cantaloupe it was slightly inferior to Yellow Cuprocide for downy mildew control and to all copper compounds for powdery-mildew control. *Phytotoxicity:* Zinc ethylene bisdithiocarbamate was nonphytotoxic to potato, tomato, and celery. It improved color on cantaloupe and cucumber but caused slight reduction in leaf size. *Yield:* Zinc ethylene bisdithiocarbamate gave the highest yield of potatoes, tomatoes, cucumbers, and cantaloupes; celery yields were not obtained. A second formulation of zinc ethylene bisdithiocarbamate (coded IN 5446) was also tested at equivalent active ingredient concentration to He 178c. It was as effective in disease control and yield response as He 178c, but was somewhat more toxic on cucumber and cantaloupe.

New organic fungicides and insecticides for potatoes. HEUBERGER, J. W., and L. A. STEARNS. Research in 1945 and 1946 on use of copper and new organic fungicides (Bordeaux, Compound A, Zerlate, Dithane plus zinc sulphate-lime, zinc ethylene bisdithiocarbamate) and insecticides (DDT, Gammexane, Rhothane, X3956) for disease and insect control showed that: (1) DDT was the outstanding insecticide for potato leafhopper control; (2) DDT was noninjurious to potatoes; (3) DDT gave high yield responses when leafhoppers were present but not when they were absent; (4) DDT has little or no fungicidal value; (5) DDT and the copper and organic fungicides were compatible since each material was as effective when used in combination as when used alone; (6) Zerlate (zinc dimethyl dithiocarbamate) gave excellent control of early blight but failed to control late blight satisfactorily;(7) Dithane (disodium ethylene bisdithiocarbamate) plus zinc sulphate-lime gave excellent control of both early and late blights and high yield response; (8) Zinc ethylene bisdithiocarbamate (reaction product-He 178c; IN 5446) gave the best control of both early blight and late blight, and the highest yield of potatoes, of any fungicide used; (9) a combination of zinc ethylene bisdithiocarbamate and DDT was the most effective combination of any used for joint disease and insect control and gave the highest yield response.

The toxicity to tomato cuttings of several microbial and other polysaccharides. HODGSON, ROLAND, W. H. PETERSON, and A. J. RIKER. The work on wilting induced by a glucosan from crown-gall bacteria has been extended to include several other polysaccharides. All these (in 0.4 per cent solution) produced in tomato cuttings a wilting, and, in addition, some caused irregular necrotic areas in the leaflets. Symptoms were of two general types: (1) a wilting and necrosis of the leaflets, and (2) a wilting primarily

of the stems. Intermediate effects were sometimes encountered. Toxicity of the first type appeared with the following: glucosan from crown-gall bacteria, inulin, soluble starch, fructosans (*Azotobacter indicum* and *Bacillus subtilis*), dextran (*Bacterium* sp.), a polysaccharide from black spruce, and three water-soluble, corn-syrup dextrins of different molecular size. Symptoms of the second type were shown by a gum preparation (*Rhizobium trifolii*), dextrans (*Leuconostoc mesenteroides, Leuconostoc dextranicum*, and an unidentified soil organism), and an amylo-dextrin preparation. Factors appearing important in the type of symptoms induced are solubility and molecular size of the polysaccharide. The water-soluble polysaccharides of low molecular weight induced wilting of the first type. Since all of these preparations were toxic, it appears that other polysaccharides may induce similar symptoms, and that wilt-inducing plant pathogens, particularly, should be examined for substances that act in a similar way.

Parasitism of Actinomyces scabies on various plants. HOOKER, W. J. Seedling plants representing 8 families developed root necrosis when grown in soil-extract agar artificially infested with pure cultures of *Actinomyces scabies*. A group of 10 cultures of *Actinomyces sp.* were tested on seedlings of wheat, garden pea, soybean, corn, radish, and cucumber, and on potato sprouts. Six of the 10 cultures caused neither appreciable necrosis of potato stems nor injury to seedling roots. The remaining 4 cultures, 3 of which scabbed potato tubers severely, caused necrosis of potato stems in a manner typical of *A. scabies*. These same four cultures caused severe necrosis of roots as well as a reduction in root weight with wheat, pea, soybean, and radish. Necrosis was most pronounced on root tips, and the development of secondary roots was almost inhibited. Corn roots were only slightly necrotic but their weight was markedly reduced, the most noticeable reaction being a slight thickening of the secondary roots. Roots of seedling plants of barley, oats, onion, tomato, eggplant, squash, red beet, carrot, parsnip, and Lima bean became necrotic when tested with one pathogenic culture of *A. scabies*. Cucumber roots were apparently unaffected by any of the 10 cultures.

Stem necrosis of potatoes caused by Actinomyces scabies. HOOKER, W. J., and G. C. KENT. Additional information is presented concerning parasitism of *Actinomyces scabies* on potato stems. Brown, necrotic lesions on subterranean Cobbler stems originating at lenticels or at points of emergence of stolons and secondary roots were obtained in greenhouse experiments using artificially infested peat soil. In advanced cases, the stem was girdled and rotted at the base with vascular discoloration extending up the stem 6 to 8 internodes. Terminal leaflets were rolled upward, chlorotic, purple to red, coloration beginning at base and progressing toward tip. Lesions caused by *A. scabies* were lighter brown than those typically caused by *Rhizoctonia solani*, with the margin not so clearly defined. In such necrotic tissue the organism consistently was demonstrated histologically and successfully isolated. In a test of 10 potato progenies, varieties resistant to tuber scab were likewise resistant to stem necrosis. In the summers of 1945 and 1946 in peat soils of northern Iowa well-developed, somewhat circular lesions were present on stems of resistant and susceptible potatoes with *A. scabies* sporulating on the surface and with its filaments well established in the tissues.

Two new fungicidal molecular configurations. HORSFALL, JAMES G., and RAYMOND W. BARRATT. The fungicidal action of 4-nitrophenyl-2,3-dichloroisobutyl ether, 4-$NO_2C_6H_4OCH_2CCl(CH_3)CH_2Cl$ (code Cr 1520) was discovered in the laboratory in 1945, and the action of 1 hydroxy-2-trichloroethyl bis-2-chloroethyl phosphite, $(ClCH_2CH_2O)_2POCH(OH)CCl_3$ (code Cr 1432), in 1946. Both were compared in the field as sprays at 2½ lb. per 100 gal. with disodium ethylene bisdithiocarbamate (code D) and 2,3-dichloronaphthoquinone (code P). Percentage control = 100 − (disease in check × 100/disease in treated) for the two doses was as follows: *Cercospora apii* in 1945—Cr 1520—68 and 32, D (1 lb. only)—97.5; in 1946—Cr 1432—42 and 39, D—92 and 88, P—94 and 92; *Septoria apii* in 1946—Cr 1432—35 and 35, D—94 and 91, P—98 and 92; *C. lindemuthianum* in 1946—Cr 1520—62 and 57, D—76 and 62, P—86 and 67; also in 1946—Cr 1432— 73 and 52, D—83 and 62, and P—80 and 42. Whether improvement in conditioning or in molecular structure will lift the performance of these new materials remains to be seen. Differences for Cr 1432 between beans and celery already suggest specificity. Thanks are due Rohm and Haas Co. for the chemicals.

An organic cadmium fungicide for turf diseases. HOWARD, F. L., and H. L. KEIL. Para-aminophenyl cadmium dilactate has given superior control of the four principal diseases of Creeping and Velvet Bent turf, namely: dollar spot (*Sclerotinia homoeocarpa*), copper spot (*Gloeocercospora sorghi*), brown patch (*Corticium vagum*), and pink patch (*Corticium fuciforme*). Comparison with 20 fungicidal chemicals in randomized replicated plots for 2 seasons has shown the cadmium complex to be nonphytotoxic, and to have unusual disease-arresting and residual fungitoxicity. Dosages of 0.1 lb. of wettable

powder containing 20 per cent toxicant in 10 gal. water (1: 4000) applied to 1000 sq. ft. have proved effective. Two applications retarded copper spot development on Piper Velvet Bent to a trace, whereas control areas developed 24 per cent disease. An application to Narragansett Creeping Bent with 7 per cent area killed by dollar spot permitted filling in by new growth within 10 days in contrast to increased disease in the nontreated area.

Vulcanized saponifiable oils (factices) as fungicide deposit builders. HOWARD, FRANK L., and HUGH H. MOSHER. A common weakness of fungicides is lack of tenacity on plant surfaces and inability to build up a sufficient deposit of the toxicant to compensate for this. Fat-sulphur condensates made by reacting saponifiable fats and oils with sulphur have been found which will permit spray residues of water-soluble, surface-active toxicants to adhere to plant surfaces despite washing. Vulcanized rapeseed, soya, corn, castor, linseed, and other vegetable oils are nonphytotoxic at effective concentrations and decrease the injuriousness of some fungicides. While these factices can be added separately to the pesticidal spray, at the rate of 0.1 lb. to 1.0 lb. per 100 gal. water, a homogenous, stable, combined solution can be obtained. DDT and similar oil-soluble insecticides can be added to the formulation. A preparation of rapeseed factice with lauryl isoquinolinium bromide as the toxicant has given an L.D. 98 at 1 p.p.m. against *Macrosporium sarcinaeforme*. The residue from a water drop containing 1: 5000 of the toxicant can be washed more than 30 minutes and still inhibit germination better than 85 per cent of *M. sarcinaeforme* spores. (Rhode Island Agricultural Experiment Station and Onyx Chemical Co.)

The effect of some of the newer fungicides on yield of potatoes in the absence of early and late blight. HOYMAN, WM. G. Using a triple lattice design with 6 replications, 6 applications of certain fungicides were applied at 10- to 12-day intervals from July 12 to September 3, 1946, to Bliss Triumph potatoes at Grafton, North Dakota. The manufacturers' recommendations were followed with respect to the concentrations of the materials. In order to reduce the damage from insects, 1 lb. of actual DDT was added to each 100 gal. spray material and the materials applied at the rate of 150 gal. per acre at 400 lb. pressure. Dusts were applied at the rate of 40 lbs. per acre and included 5 per cent of actual DDT. In the absence of early and late blights, the dust treatments and their respective yields in bushels per acre were as follows: He 178, 165; Copper A, 147; Zerlate (zinc dimethyldithiocarbamate), 169; Tribasic Copper Sulphate, 156; DDT, 162 and DDT, 166. The various materials used as sprays with their respective yields were: Phygon (2,3-dichlor 1,4-naphthoquinone), 164; Tribasic Copper Sulphate, 162; Bordeaux Mixture, 172; Dithane D 14, 168; He 178, 154; Polyethylene Polysulphide (Omilite), 133; Polyethylene Polysulphide plus Phygon, 177; Zinc Ethylene Bisdithiocarbamate, 183; and Manganese Ethylene Bisdithiocarbamate, 153. The plots receiving no treatment yielded 138 bushels per acre.

Structures corresponding to appressoria and substomatal vesicles produced on nutrient-solution agar by germinating urediospores of cereal rusts. HURD-KARRER, ANNIE M., and H. A. RODENHISER. Germ-tubes from urediospores of *Puccinia graminis tritici*, *P. triticina*, *P. dispersa*, *P. coronata avenae*, *P. hordei* (simplex), and *P. sorghi* on the surface of agar made with a mineral nutrient solution and glucose produced bodies whose mode of development, size, and shape identify them as structures corresponding to appressoria and substomatal vesicles. They had, for the most part, the size, distinctive shape, septation, and number of infection hyphae that characterized the substomatal vesicle of the particular species as described by Pole-Evans from stained sections of their respective host plants. Infection hyphae were never observed to develop beyond a length of about 300 μ.

Graft-transmissible brooming disease of walnut. HUTCHINS, LEE M., and HORACE V. WESTER. Brooming of black walnut (*Juglans nigra*), butternut (*Juglans cinerea*), and Japanese walnut (*Juglans cordiformis* var. *ailantifolia*) has been observed over several years in parts of the eastern United States. It is characterized mainly by brooms or sucker growth on main stems and branches, tufting of terminals, profusion of branchlets from axillary buds, dwarfing of leaves, and sometimes by death of the trees. Symptoms vary from mild to severe on different trees of these species and are particularly pronounced on Japanese walnut. Inoculation experiments employing patch-bark grafts, performed in 1944 and 1945, demonstrated transmissibility of the brooming disease in black walnut and in Japanese walnut, the incubation period varying from several months to two years, under conditions of the experiments. Cross inoculations among *Juglans* species are not sufficiently mature for a report at this time. Transmission of the brooming disease by grafting and absence of a visible pathogen indicate probable virus causation. (U. S. Department of Agriculture and U. S. Department of the Interior.)

Virus attenuation and mutation. JOHNSON, JAMES. Sea-holly (*Eryngium aquaticum*) inoculated with ordinary tobacco mosaic virus, which yields severe symptoms on tobacco, results in only mild symptoms on tobacco after passage through sea-holly. Single-lesion strains of the severe and mild forms of the disease may be isolated and pure-lined on *Nicotiana glutinosa* or the *glutinosa-tabacum* hybrid, by repeated dilution. The severe strain usually produces a large spreading lesion and the mild form a smaller and more localized lesion on the latter hosts. The severe strain is, however, more localized and slower moving in the sea-holly than is the mild strain. Thus this host acts as a "filter" host separating the two related strains in question, and yields what is commonly referred to as an attenuated strain without any true mutation occurring in the sea-holly. Single-lesion severe strains have, however, been changed so as to yield mild forms by growing the virus on tobacco at a temperature of 36–37° C. for several days. The latter is regarded as mutation to a mild form which can be isolated either by the local lesion method or by passage through a host such as sea-holly, yielding a pure attenuated virus. Thus mutation of the tobacco mosaic virus normally precedes attenuation. The terms "attenuation" and "mutation" are frequently used loosely in the literature; whereas they be quite definitely applied in the results with *Eryngium*, when tested by the pure-lined single-lesion method.

Fungus invasion of water-congested tissues. JOHNSON, JAMES. Several plant species were grown in the greenhouse on sandy soils low in available potash. Part of each series was transferred outdoors for 5–10 days. The indoor and outdoor plants were then inoculated with typical fungus parasites by atomizing without wounding. They were placed in a moist chamber with controlled soil and air temperatures favoring water-congestion in the leaves. The moist-chamber exposures usually varied between 12 and 24 hours. The outdoor plants commonly water-congested first and most extensively and were usually more rapidly and heavily infected by the fungus parasites than greenhouse-grown plants. Frequently, susceptible varieties water-congested more easily than resistant varieties grown in the same container. When resistant varieties became congested, they often developed heavy infection. The results varied with the time required for congestion and allowed for spore germination and infection. All factors concerned could not be controlled at will, and some uncertain results were obtained. The best correlations were secured with bean anthracnose, tomato late blight, leaf rust of oats, and leaf rust of wheat. The results with potato late blight and sunflower rust were more difficult to interpret because of failure to secure good visible signs of water-congestion.

The effect of hydrogen-ion concentration on the toxicity of Spergon. KELMAN, ARTHUR. A relationship between hydrogen-ion concentration and fungitoxicity of Spergon (98 per cent tetra-chloro-p-benzoquinone or chloranil) was demonstrated in the laboratory. Mycelial development of *Glomerella gossypii* and *Rhizoctonia solani* in nutrient solutions containing varying amounts of Spergon was suppressed at pH 3.8–4.8, whereas growth developed at pH 6, 7, and 8, reaching a maximum at pH 7. In order to obviate shifts in pH, discs of mycelium were exposed to suspensions of Spergon in buffer solutions adjusted to 6 pH levels. When the discs were removed to potato-dextrose-agar plates, slowest mycelial development occurred from discs taken from the most acid buffer solutions. In these tests the action was fungistatic rather than fungicidal. A distinct pink coloration usually developed above pH 4.8 in solutions containing Spergon, increasing in intensity as the pH level increased. In greenhouse experiments to determine if the phytotoxicity of Spergon in soil was influenced by hydrogen-ion concentration, the toxic effect on cotton seedlings was greatest in the most acid soil. The decrease in effective toxicity of Spergon with decrease in hydrogen-ion concentration is probably associated with the conversion of chloranil to a less toxic compound. Although variation in effective toxicity may occur in a normal pH range, the low solubility of chloranil precludes complete conversion or complete loss of toxicity.

Overwintering and infection of the tomato anthracnose organism. KENDRICK, J. B., JR. From a tomato field at Madison, Wisconsin, heavily infected with *Colletotrichum phomoides*, dead stems and petioles collected in late fall and early the next spring had inconspicuous black stromatic bodies that readily yielded the causal organism, showing that the latter overwinters in tomato refuse. Plants grown on the same field the following season were inoculated with a nonpigmented strain, isolated in Wisconsin in 1942 by W. J. Hooker, which served as a marker. Random isolation from affected fruits showed that practically all infection came from overwintering refuse and not from current season artificial inoculation. Greenhouse studies showed that the organism can infect the foliage of young tomatoes, peppers, and eggplants in a very humid atmosphere. At 28° C. lesions developed on tomato seedlings after 3 days of constant moisture, while 11 days were required at 16°. Further development of infection after the abscission of the infected cotyledons and leaves did not occur even at continued high moisture.

Current season infection of tomato foliage appears to be not so important in causing fruit infection as does the primary inoculum from tomato refuse of previous seasons.

Quick decline of orange trees. KLOTZ, L. J., and G. A. ZENTMYER. Quick decline has, with certainty, been found attacking only sweet orange trees (2 to 50 years of age) growing on sour orange rootstocks in light sandy soil of San Gabriel Valley, California. General appearance of an affected tree is similar to that caused by girdling. First symptom noticeable in top is dull, ashen color, and curling of leaves lengthwise and upward. Leaves may gradually drop in chronic cases, or suddenly wilt and dry in place on tree in acute cases of collapse. Death of fibrous roots at periphery of root system precedes top symptoms and progresses inward toward trunk, involving fibrous and larger roots. In chronic form, dropping of leaves and twig dieback keep pace with root destruction, although trees may put out new, feeble growth of foliage and roots, and continue to live. Starch disappears in affected roots and trunk, but reappears in limited amounts following new leaf growth. There is also less sugar and slower respiratory and catalase activity in affected than in healthy trees. Sieve-tube degeneration is invariably found in the sour orange stock of mature declining trees. Histological symptoms resemble those found in sweet cherries growing on Mahaleb roots and affected by Green Valley Buckskin virus.

Late wilt of flax. KOMMEDAHL, T., and J. J. CHRISTENSEN. During the last decade, several thousand lines and varieties of flax were tested annually for wilt resistance on flax-sick soil. Varieties that differ greatly in susceptibility differ also in time of wilting. Newland and Punjab tend to wilt as seedlings; Linota and C.I. 423 tend to wilt late in the season; others, like Pale Pink, Redwing, and Crystal, are variable. In general, progenies from crosses involving Newland tend to wilt early; whereas progenies containing C.I. 423 tend to wilt late. These tendencies, probably genetic, are influenced by environment, especially temperature. Punjab, wilting as seedlings in most seasons, wilted late in the cool season of 1945. The fact that varieties wilt at different times during a season is significant in varietal and inheritance studies. In late wilt, partial infection of the plant sometimes occurs. *Fusarium lini* ordinarily may be isolated from both susceptible and resistant plants. The fungus may be present internally long before wilting occurs; but the entire vascular system must be invaded before the plant succumbs. In partial wilt one side of the stem may be brown, while the other side remains green; but *F. lini* may be isolated only from the vascular bundle of the discolored side.

Seed transmission and suggested control measures for stripe smut of timothy. KREITLOW, K. W. Seed harvested from smutted plants of timothy yielded 5.8 per cent infected plants among the 877 seedlings observed. The infection originates from chlamydospores borne on the surface of the seed. When 550 contaminated seeds were surface-sterilized in 1: 500 mercury bichloride solution, no smutted plants developed. Among the 500 control seedlings, 3.5 per cent were infected. When seeds obtained from smutted plants were stored 1 year at room temperature, no smutted plants developed among 343 seedlings observed. Tests with dust fungicides are underway.

A mosaic disease of Mohawk potato caused by a virulent strain of the latent-mottle virus. LARSON, R. H. A virus causing a pronounced mosaic mottle in Mohawk has been found to be a severe strain of the potato latent-mottle group. The relationship was established by cross-immunity inoculations on tobacco with the potato ring-spot virus, by physical properties of plant extract *in vitro*, by serological precipitin reactions as well as precipitin-absorption reactions, and by the production of lethal streak on tomato by mixed infections with the virus of ordinary tobacco mosaic. The potato seedling 41956 is immune. Repeated aphid (*Myzus persicae* and *Macrosiphum solanifolii*) transmission tests have yielded negative results. The virus was readily transmitted artificially by plant extract to *Capsicum annuum, Cyphomandra betacea, Nicotiana rustica, N. glutinosa, Datura stramonium* var. *tatula, D. metel, Nicandra physalodes, Solanum aculeatissimum,* and *Schizanthus retusus.* Inoculated *Nicotiana tabacum* (var. Connecticut Havana No. 38 and White Burley) developed a very pronounced mottle with no evidence of ring lesions or line patterns. Graft transmission to the potato variety Epicure resulted in lethal top-necrosis whereas only a mottling and crinkling developed on the varieties President and British Queen. *Lamium hybridum* (Labiatae) developed a diffused systemic mottling when infected. (University of Wisconsin and U. S. Department of Agriculture.)

Perennial groundcherries as overwintering hosts of the potato yellow-dwarf and veinbanding viruses. LARSON, R. H. *Physalis virginiana* and *Physalis heterophylla* are common weeds in the potato fields in central Wisconsin. Many plants have been found that are dwarfed and have rolled and vaguely mottled leaves. Transmission tests with

nonviruliferous adults of the clover leafhopper (*Aceratagallia sanguinolenta*) to the potato variety Green Mountain resulted in the recovery of the yellow-dwarf virus from naturally infected plants of both species. Transmission tests with *Myzus persicae* to potato, *Nicotiana tabacum*, and *Hyoscyamus niger* also resulted in the recovery of the veinbanding virus. In the late fall, when most vegetation has been killed by frost and is dead, the common perennial *Physalis* remains green for a longer period, usually until late in November, affording important feeding plants for both leafhoppers and aphids. The late growing season also lengthens the period for the viruses to become established in the perennial roots of *Physalis*, and these thus become an important reservoir for and a means of overwintering of these 2 potato viruses. (University of Wisconsin and U. S. Department of Agriculture.)

Ethylene dibromide—a promising new soil fumigant. LEAR, BERT. Ethylene dibromide has given excellent control of the root knot nematode, *Heterodera marioni*, in replicated greenhouse and field trials. Preliminary tests made in glazed, gallon crocks of infested soil showed that 0.2 cc. of the undiluted chemical was lethal to all forms of the nematode. For larger trials in greenhouse ground beds and field plots, the fumigant was diluted with propylene dichloride at the rate of 1 to 9 by volume. A commercial product, Dowfume W–10, found to be equally effective, contains an inexpensive naphtha fraction as the diluent in place of the propylene dichloride. Almost complete eradication of the nematode has been achieved with 4 cc. of the 10 per cent mixture applied 4 inches deep at staggered 10-inch intervals in rows 10 inches apart. Approximate cost per acre at this dosage rate is $75. The fumigant has successfully penetrated within 24 hours solid, sound tomato root galls in soil. Control has been equally good with and without a water seal. Its low vapor pressure causes it to persist in a sandyloam soil, to the detriment of tomato transplants, for a period of 1 to 2 weeks at a soil temperature of 70° F. Limited trials have indicated its poor fungicidal properties.

The effect of an antibiotic substance on apple leaf infection by Venturia inaequalis. LEBEN, CURT, and G. W. KEITT. A species of *Streptomyces* was found antagonistic on agar to all of 29 phytopathogenic fungi tested, and not antagonistic to most bacteria, including those commonly used in antibiotic assays and certain phytopathogens. For the preparation of concentrates of the antibiotic substance, the antagonistic organism was grown in shake-flasks containing a corn-steep-glucose medium. The active material was obtained by ethanol extraction of the precipitate formed when the culture filtrate was acidified with HCl to pH 2.5. Further fractionation yielded a solution completely inhibiting growth of *Venturia inaequalis* at 1:8,000,000 and *Sclerotinia fructicola* at 1:11,000,000 (solids basis, agar-streak test method). There was no apparent loss in activity of ethanol solutions stored 11 months at 8° C. The active material is precipitated from ethanol solutions on the addition of water. In 3 greenhouse tests, infection was prevented or greatly reduced on susceptible apple leaves by a single spray application of an ethanol solution of the active material 4 hours or 4 days prior to inoculation with *V. inaequalis*.

Field resistance to leafroll infection in potato varieties. LOCKE, SETH BARTON. Twenty-three potato varieties and numbered seedlings were grown at 3 locations in Washington State in 1944 and 1945, exposing them to natural leafroll infection. The percentage of plants infected was determined by replanting samples from each plot and observing the amount of tuber-borne disease. Variations occurred in the relative performance of individual varieties at the 3 locations and during the 2 seasons, but the combined data show that the varieties form an almost continuous series with respect to amount of leafroll infection. At the most resistant end of the series are Katahdin (11 per cent) and Sequoia (18.5 per cent). The most susceptible are Netted Gem (51 per cent), Chippewa (54 per cent), and Burbank (58 per cent). Marked differences in amount of leafroll infection at the different locations are correlated with differences in recorded temperature and humidity. These weather factors appear to act through their effects upon migration of the aphid vectors.

Nutritional sprays on grapes. MACK, G. L., and N. J. SHAULIS. The addition of urea to foliage sprays as a means of controlling plant nutrition was investigated. Grape foliage was sensitive to urea, being severely injured by a single application of 4 lb. per 100 gal. The injury is of 2 distinct types, depending on whether the spray is applied to the upper or lower side of the leaf. Urea applied only to the upper surface caused marginal necrosis. Urea applied only to the under surface caused $\frac{1}{4}$-inch rounded lesions distributed over the whole surface. Copper in the form of Bordeaux mixture (3–3–100) prevented injury from urea up to a concentration of 4 lb. per 100 gal. Higher concentrations of 8 and 12 lb. urea in Bordeaux caused proportionally greater injury within 5 days. At harvest, leaves sprayed with 4, 8, and 12 lb. urea were progressively greener

than those sprayed with Bordeaux alone. Analysis of the blade for total nitrogen showed no significant differences, indicating that nitrogen from foliage sprays is not accumulated in the leaf.

Spraying and dusting rutabagas to prevent water-core (boron deficiency). MAC-LACHLAN, J. D. Water-core (boron deficiency) of rutabagas can be prevented by a single foliage application of borax, as a spray or as a dust, when the rutabaga root is 1 to 1½ inches in diameter. The spray contains 12 lb. of fine borax, 3 lb. of bentonite clay and ¼ cup (or less) of Orthex per 40 gal., Imperial Measure. A pressure sprayer with 3 nozzles per row, applying 40 to 50 gal. per acre, is recommended. The dust contains fine borax (about 300 mesh) plus the carrier Celite, mixed in equal proportions by weight; the application of 40 to 50 lb. of this mixture per acre is recommended. Experimental results have been substantiated through commercial application by growers. Spraying and dusting are now recommended in those districts of Ontario where soil applications of borax fail to give results.

Differentiation of cultural types of Sclerotinia spp. by means of hydrogen-ion concentration. MADER, E. O., and M. N. TELLER. Sixty-two isolates of *Sclerotinia spp.*, which appeared identical on nonacidified potato-dextrose agar (pH 5.6–6.0), could be classified into 12 distinct cultural groups when grown on potato-dextrose agar adjusted to a pH range of 3.1–3.7 with additions of lactic, tartaric, malic, or citric acid, or Sorensen's sodium citrate-hydrochloric acid buffer solution. Monosporous isolates from a single apothecium and even from a single ascus did not necessarily fall within the same cultural type. The cultural types obtained from 5 ascospores isolated from a single ascus fell into three cultural groups. When representatives of the various cultural types were tested again after 8 and 12 months, they fell into the same cultural groups, although topography of types varied slightly.

The pea-seed-treatment method of evaluating fungicides in the greenhouse. McCALLAN, S. E. A. Perfection peas are treated with experimental chemical dusts on a percentage seed-weight basis, rolled and planted in 10 seed-row units in flats or greenhouse benches of naturally infested soil. Rows are replicated several times in randomized incomplete blocks or other arrangements. Observations are taken weekly for one month on emergence and stand, the difference being post-emergence damping off. Emergence is completed in two weeks but post-emergence damping off continues with further time. In general, there is no evidence that seed protectant fungicides control post-emergence damping-off expressed on absolute basis of seeds planted. The regression of height of seedlings on percentage of stand is highly significant. Dosage response curves are very flat. Increasing depth of planting delays emergence but has little final effect. Infested soil may be diluted considerably with good soil without appreciably diminishing the amount of seed decay. Used soils are composted with the pea plants, later mixed with good soil for further tests. Block effects usually are not significant. Treatment × test interaction is but little greater than replicate error. Differences of 16 per cent can be demonstrated on the basis of 50 seeds per treatment for 2 tests.

The isolation of Pythium from soil at various seasons of the year as related to soil temperature and moisture. McLAUGHLIN, J. H. Pythium has been isolated from the surface, 3-, 6-, 9-, 12-, 15-, and 18-inch soil levels at all seasons of the year. Isolations were made at approximately weekly intervals by placing minute quantities of soil beneath 2 per cent water agar in Petri plates. Soil-temperature and moisture data were taken concurrently. The percentages of Pythium isolates were generally high in the winter, spring, and fall seasons and low in the summer. A combination of high soil temperature with low moisture generally resulted in a reduction in the percentage of Pythium isolates. Although the percentage of Pythium isolates obtained under some environmental conditions reached very low levels, seedling tests showed that *Pythium* was not absent from the soil. The corelationships between soil temperature, moisture, and percentage of Pythium isolates were distinct and consistent to such a degree that the latter could be estimated with considerable accuracy from determinations of only the first 2 variables.

Factors influencing sporulation of Sclerotinia fructicola, Venturia inaequalis, and Phytophthora infestans. MILLER, H. J. Factors favoring growth of a fungus in culture do not necessarily produce optimum sporulation. High yields of spores are desirable for slide-germination tests as well as for artificial inoculation of plants, both in greenhouse and in field. Knowledge of optimum conditions for spore production should be useful for interpreting epiphytotics, since it is the dissemination of spores which accounts, in large part, for the distribution of diseases in an area. *Venturia inaequa*- gave highest yield of conidia on 10 per cent malt agar at 18° C. Time required

optimum yield was 25 days at 9° C.; 18 at 12° C.; 11 at 15° C.; 11 at 18° C. and 7 at 21° C. with a marked drop after these periods in number of spores obtainable in a water suspension. *Sclerotinia fructicola* gave higher yield on malt than on potato-dextrose agar with maximum number of conidia being produced on ten per cent malt agar. *Phytophthora infestans* produced highest number of sporangia at 18° C. in 21 days on Lima-bean agar. Addition of Brewer's yeast, yeast extract, riboflavin, thiamin chloride, and corn steep liquor in varying concentrations to potato-dextrose agar did not increase sporangial yield.

Heat treatments of sour cherry carrying yellows and necrotic ring spot. MOORE, J. DUAIN. In greenhouse studies in 1944 bud sticks from Montmorency trees affected with both yellows and necrotic ring spot were treated in a water bath at 50° C. ± 0.1°. The bud sticks were 15 cm. long and 2.7–3.0 mm. in diameter at the base. The periods chosen for treatment were from 2 to 16 minutes at 2-minute intervals and from 17 to 22 minutes at 1-minute intervals. Two bud sticks were removed from the bath at each interval, and 1 bud from each stick was budded into each of 2 Montmorency trees. Two trees were budded from sticks receiving no treatment, and 2 were left unbudded. In 1944 all budded trees, except 1 budded with untreated buds, showed necrotic ring-spot symptoms, and by the spring of 1946 all budded trees, except 4 that died, showed yellows symptoms. The nonbudded trees remained healthy. In a limited experiment with potted trees subjected at bud break to heat treatment of 35° C. for 11, 19, 28, and 35 days, respectively, yellows symptoms appeared the following year on diseased trees treated for 11, 19, and 28 days, respectively, while the tree treated 35 days died. All control trees remained healthy.

Temperature and seasonal development of host in relation to expression of leaf symptoms of sour cherry yellows. MOORE, J. DUAIN, and G. W. KEITT. Eighteen potted Montmorency trees in the greenhouse were budded in 1944 shortly after bud-break with buds from a Montmorency tree affected by yellows. Before budding, all trees were incubated at approximately constant temperature of 24° C. for 9 days. Immediately after budding, 3 trees were placed at appromixately constant temperature of 16° C. Twelve were transferred in groups of 3 trees each to 16° C. after additional periods at 24° C., as follows: (1) 1 week after budding (full bloom); (2) 2 weeks after budding (petal-fall); (3) 3 weeks after budding; and (4) 4 weeks after budding. Three trees were kept at 24° C. No yellows symptoms occurred in 1944. All trees were carried out-of-doors through the summers of 1944 and 1945. In the greenhouse seasons of 1945 and 1946, all trees were forced to break dormancy at 24° C. and each tree was handled in the same manner as in 1944. Both in 1945 and 1946, abundant leaf symptoms of sour cherry yellows developed on all trees transferred to 16° C. shortly after bud-break and on those transferred in bloom. In 1945 no other trees showed yellows symptoms, but in 1946, 1 tree transferred at petal-fall had 2 yellows leaves.

A technique for making serial dilutions. MOORE, M. B., and T. KOMMEDAHL. A hypodermic syringe fitted with a 6-inch delivery tube is used to dispense accurately and aseptically known volumes of a medium to culture tubes in a dilution series. Using a sterile syringe and sterile, plugged culture tubes, a known volume of medium, say 10 cc., is added aseptically to each tube of a series. Ten cc. of a stock solution, such as a fungicide, are then added to the first tube of the dilution series. Thorough mixing is insured by manipulating the plunger of the syringe several times, while the delivery tube is inserted in the culture tube. A 10-cc. portion of the first dilution is then drawn up, delivered into the second tube, and mixed. Similar dilutions continue throughout the series. Each tube of such a series is one-half the concentration of the preceding one. Other ratios, like 1:3:9, 1:4:16, and 1:10:100, may be used. In 890 tubes of serial dilutions only 5 contaminations occurred. Simplicity and ease of manipulation are merits of the technique.

Production of mint species hybrids resistant to verticillium wilt. NELSON, RAY. Verticillium wilt of peppermint and spearmint is very destructive in Michigan, occurs in Indiana and is now appearing in Oregon and Washington plantings of peppermint. It has caused the abandonment of thousands of acres of the best mint soil in Michigan and has driven the industry from areas where the highest quality oil was formerly produced. Peppermint (*Mentha piperita*) is a complex hybrid, is almost completely sterile, and flowers normally 3–4 weeks later than the spearmints (*Mentha spicata*). Beginning in 1942, methods have been developed for producing species hybrids, using a noncommercial variety of *Mentha spicata* as the pollen parent. This plant is highly resistant to wilt and, although a hybrid, produces good pollen abundantly. All attempts at hybridization by hand-pollinations failed. Utilizing the positive photoperiodic response of *Mentha piperita* to supplementary illumination, delayed plantings and other methods to delay

flowering of the pollen parent, mass insect pollinations have resulted in the production of species hybrids highly resistant to wilt. The hybrids are of diverse types; the most desirable ones mature early and are highly productive of oils of good quality.

The specific pathogenesis of the Verticillium that causes wilt of peppermint. NELSON, RAY. *Verticillium albo-atrum* is reported as the cause of wilt in many different suscepts, including ornamental, fruit, vegetable, and fiber plants, ornamental and fruit trees and shrubs. Cross inoculations with isolates from unrelated host plants are usually successful. The fungus is thus characterized by a nonspecific pathogenesis. *Verticillium dahliae* has been isolated from a number of unrelated plants and successful cross inoculations have been reported. The fungus that causes the destructive wilt of peppermint is morphologically similar to *Verticillium dahliae*. In soils at maximum levels of natural field infestation and in those artificially infested with very virulent isolates and maintained at temperature and moisture levels most favorable for wilt it has not infected plants highly susceptible to isolates of *Verticillium albo-atrum*. Conversely, isolates of *Verticillium albo-atrum* from cotton, pepper, okra, eggplant, snapdragon, chrysanthemum, blackberry, maple, and other very susceptible plants have not infected peppermint under the most favorable environal conditions for wilt. The specific pathogenesis of the mint fungus for plants in the genus *Mentha* and very closely related genera, indicates the existence of dissimilar strains within the *Verticillium dahliae* group.

Effect of dust composition on adherence of copper dusts. NIKITIN, A. A. It was found that chemical composition, structure of diluents, and their rate of oil sorption have profound effect upon adherence of copper dusts treated with oil. In studying the improvement of adherence and delivery of dust it was found necessary to determine the effect of oil content of the dust. In the method of application, pressure and distance from target were of special interest. Copper dusts, containing Pyrax, Loomkill, and Eastern Magnesia, were subjected to adherence tests by varying oil content, pressure, and distance from target. Pyrax, containing 2 per cent oil, gave better adherence than Eastern Magnesia and Loomkill upon decreasing distance from target and reducing pressure. However, Pyrax, containing 1 per cent oil, gave better adherence upon increasing distance from target and reducing pressure. Eastern Magnesia, containing 1 and 2 per cent oil, gave better adherence upon increasing pressure and reducing distance from target. Substantial improvement in adherence was secured with Loomkill, containing both 1 and 2 per cent oil, upon increasing pressure and reducing distance from target.

Actinomyces and bacteria antagonistic to Actinomyces scabies. ORELLANA, RODRIGO. The growth of *Actinomyces scabies*, the cause of potato scab, is influenced by the antibiotic effect of other soil actinomyces and soil bacteria. On potato-dextrose agar plus peptone, at pH 7.0, the growth of *Actinomyces scabies* was suppressed by *Actinomyces griseus* (obtained originally from S. A. Waksman) by an isolate of the *griseus* type obtained from Waukegan silt loam, by a blue-pigmented *Actinomyces*, by an isolate from lime-treated soil, by three isolates from sulphur-treated soil, and by one isolate from manured soil. All these isolates, except *Actinomyces griseus*, were obtained at University Farm, St. Paul, Minnesota. *Actinomyces scabies* also was suppressed by *Bacillus mesentericus* obtained from potato-scab lesions at St. Paul, and 4 isolates of *Pseudomonas spp.* (soil isolates obtained from Dr. C. E. Skinner of the Department of Bacteriology, University of Minnesota). When isolates of *Actinomyces scabies* from a single potato-scab pustule were grown together in culture, some isolates were antibiotic to others.

Soil and foliage applications of nitrogen in relation to apple-scab control. PALMITER, D. H. McIntosh apple trees that received annual soil applications of Uramon (urea) increased in both yield and susceptibility to scab infection as the rate of application was increased. Plots that received no nitrogen had the lowest yields and the least scab. Trees in plots that received only foliage applications in the form of Uramon 5–100 in combination with sulphur and arsenate of lead in the petal-fall and two subsequent sprays maintained yields comparable to those of the soil-treated plots and yet were more resistant to scab. For example, in 1943 plots sprayed with flotation sulphur paste 10–100 averaged 6, 8, 11, and 13 per cent fruit scab on trees that received soil applications of 0, 2½, 5, and 7½ lb. Uramon, respectively, compared with 7 per cent infection on trees sprayed with Uramon. Similar data were obtained in 1944 and 1945. The differences in scab control in the various plots were more pronounced when less effective fungicides were used. The probable explanation for the reduced susceptibility to scab in the plots that received foliage applications of nitrogen rests in the fact that these trees were at a lower nitrogen level during the early part of the season, which was the most critical period for scab control.

Epidemiology of rust in western Canada as influenced by the introduction of stem-rust-resistant varieties. PETURSON, B. Stationary slide exposures have been made at 3 places in Manitoba each summer since 1925. These show that there has been a considerable reduction during the early part of the season, and a very marked reduction during the latter part of the season, in the numbers of stem-rust spores in the air over Manitoba, as compared with the numbers present in corresponding periods during several years prior to 1939, when susceptible varieties were generally grown. Owing, no doubt, to the fact that some of the stem-rust-resistant varieties thus far released are susceptible to leaf-rust, there has been no marked change, since their introduction, in the number of leaf-rust spores caught on slides. As a result of this reduction in stem-rust inoculum the northward and westward spread of stem rust, as well as the severity of infection, as indicated by infection on susceptible varieties in rust nurseries in western Canada, has been greatly restricted. The growing of resistant varieties has produced no change in the physiologic races of wheat stem rust occurring in western Canada. However, certain formerly rare races of oat-stem rust, to which the new resistant oat varieties are susceptible, have greatly increased in prevalence, whereas the formerly predominant races have decreased.

The effect of leaf rust (Puccinia triticina) on the yield, grade, and quality of wheat. PETURSON, B., MARGARET NEWTON, and A. G. O. WHITESIDE. In field tests carried out at Winnipeg in 1944, 1945, and 1946, to determine the effect of leaf rust (*Puccinia triticina* Eriks.) on yield, grade, and quality, leaf rust infection ranged from an average of 22 per cent on some varieties in the rusted plots to 87 per cent on others, while practically no rust developed in the check plots that were protected from rust by applications of sulphur dust. Heavy infection reduced yield of seed by as much as 40 per cent, kernel weight by as much as 27 per cent, and bushel weight by from 1.4 to 3.5 pounds. Infection ranging upwards of 75 per cent reduced grades by one commercial grade, while light to moderate infection caused no grade reduction. The 1944 and 1945 samples only have been subjected to quality tests. These showed that leaf rust reduced the protein content of the seed of all the varieties tested. The reductions in seed protein ranged from 0.73 per cent to 1.50 per cent, and were statistically significant. Although lower in seed protein, the rusted samples generally produced loaves of greater volume than corresponding nonrusted samples. Apparently the rust, in some as yet unexplained manner, improved the quality of the gluten of the rusted samples. Leaf rust generally reduced flour yield slightly and increased carotene content.

Control of aster yellows in carrots by control of Macrosteles divisus with DDT. POUND, GLENN S., and R. KEITH CHAPMAN. In recent years carrot fields in southeastern Wisconsin have been heavily infected with the aster yellows virus, often as high as 75 per cent. In 1946 wettable DDT spray (2 lb. per 100 gal.) was applied on a commercial field at 100 gal. per acre and 400 lb. pressure. Six spraying dates were selected at 10-day intervals, beginning when carrots were 4 to 6 inches high. Treatments consisted of the following schedules of spraying dates: (A) all 6 dates; (B) first, third, and fifth dates; (C) first, second, and third dates; (D) fourth, fifth, and sixth dates; (E) first, second, and sixth dates; (F) nonsprayed check. No significant difference was found in amount of disease or in yield between treatments A, B, C, and E. In each the percentage of diseased plants was significantly less, and the yield significantly greater than those of treatments D and F. The differences in disease and yield of D and F were not significant. In treatment A, yellows was 38 per cent less and yield 4 tons per acre greater than treatment F. Vector counts made after each application showed that disease reduction was in proportion to insect control.

Lack of correlation between yield and disease control with fungicides. RICHARDS, M. C. Organic and inorganic fungicides were applied to tomato and potato plants to control Alternaria blight. The highest yields of marketable fruits or tubers were not always obtained from the plots showing the least defoliation. The fungicides are listed in descending order of disease control, and numbers in parentheses give the rating with respect to yield of marketable tomatoes or potato tubers. The asterisk indicates the addition of 2 lb. 50 per cent DDT. On tomatoes in 1946 the order was: 1. Bordeaux mixture, 6–6–100 (7); 2. Zinc dithane (zinc ethylene bisdithiocarbamate), 2–100 (2); 3. Tribasic copper sulphate, 3–100 (6); 4. Phygon (2,3-dichloro-1,4-naphthoquinone), 2–100 (4); 5. Zerlate (zinc dimethyldithiocarbamate), 2–100 (3); 6. Manganese dithane (manganese ethylene bisdithiocarbamate), 2–100 (5); 7. Fermate (ferric dimethyldithiocarbamate), 2–100 (1); 8. Check (8). On Green Mountain potatoes the order was: 1. Zerlate, 2–100* (3); 2. Zinc dithane, 2–100* (3); 3. Bordeaux mixture, 10–10–100* (5); 4. Bordeaux mixture, 10–5–100* (4); 5. Phygon, 2–100* (10); 6. Tribasic copper sulphate, 3–100* (6); 7. Manganese dithane, 2–100* (1); 8. DDT (dichlorodiphenyltrichloro-ethane), 2–100 (7); 9. Calcium arsenate, 4–100 (9); 10. Check (8). On Irish

Cobbler potatoes the order was: 1. Zinc dithane, 2–100* (3); 2. Bordeaux mixture, 10–5–100* (7); 3. Bordeaux mixture, 10–10–100* (4); 4. Phygon, 2–100* (5); 5. Tribasic copper sulphate, 3–100* (1); 6. Zerlate, 2–100* (6); 7. Manganese dithane, 2–100* (2); 8. DDT, 2–100 (8); 9. Calcium arsenate, 4–100 (9); 10. Check (10). The lack of correlation between yield and disease control occurred with both the organic and inorganic fungicides.

The influence of temperature and humidity on the development of white pine blister rust on Ribes leaves. RIKER, A. J., T. F. KOUBA, and B. W. HENRY. The influence of environment on infection of *Ribes* by *Cronartium ribicola* has been studied both in the greenhouse and at the Wisconsin blister-rust nursery, where white pines are being selected for rust resistance. Readings were made at intervals on the percentage of Ribes leaf area covered by uredia, necrosis, telia, and total infection. Uredia developed on *Ribes nigrum* at constant temperatures of 16°, 20°, 24°, and 28° C. With increasing temperatures, incubation time was shorter; area involved was larger; subsequent leaf necrosis was greater. However, telia developed as follows: abundant at 16°, good at 20°, occasional at 24°, and none at 28° C. The amount of infection on leaves, kept in a fog for 5 to 12 hours, increased progressively with time. The amount of infection on R. cynosbati, R. missouriense, and R. nigrum in the nursery and on R. americanum, R. glandulosum, R. hirtellum, and R. triste nearby was observed in detail during the seasons of 1942, 1943, 1944, and 1945. The development of rust in the nursery correlated closely with that at corresponding temperatures in the greenhouse. These results help to clarify the observations (1) that telia seldom develop well on most species during hot weather, and (2) that hot weather improves the chances for white pine to escape infection. (University of Wisconsin, Wisconsin Conservation Department, and United States Department of Agriculture cooperating.)

Evidence of fusion bodies from urediospore germ tubes of cereal rusts. RODENHISER, H. A., and ANNIE M. HURD-KARRER. Germ tubes from urediospores of *Puccinia graminis tritici*, *P. triticina*, *P. hordei (simplex)*, *P. dispersa*, *P. coronata*, and *P. sorghi* growing on agar containing mineral nutrients and glucose in darkness at 10° to 25° C. have been observed to form what seem to be fusion bodies. The tip of a germ tube, growing aerially, becomes suddenly distended into a spherical body and the hyphal contents crowd into it. When first formed, it is approximately the size of the urediospore. The supporting hypha soon becomes flaccid, bends, and the fusion body may contact another germ tube. There was some evidence of a breakdown in the germ-tube wall at the point of contact, and in one instance the movement of the contents of the contacted germ tube into the fusion body was observed. These bodies in a later stage occasionally produced as many as 5 hyphae, which generally failed to develop beyond about 50 μ. The number of fusion bodies increased with increasing concentrations of glucose up to 6 or 8 per cent. Formation of the bodies was completely inhibited behind a blue filter that transmitted wave lengths of from 4000 to 5400 A units, whereas behind red and yellow filters the bodies formed as in darkness.

Helminthosporium blight of oats in Arkansas. ROSEN, H. R. For several years an Helminthosporium disease of oats has been noted and studied which involves seedling blight, blighting of tillers of varying age, and localized blade and sheath spots. Under field conditions the disease up to the present has been noted only on varieties with Victoria parentage, while under artificial conditions in the greenhouse the disease has been produced not only on Victoria derivatives but also on a few varieties that have no such parentage. While spore measurements seemingly do not permit sharp differentiation of this pathogen from *H. avenae*, symptoms and varietal range differ markedly. Seed treatment with ethyl mercury phosphate preparations gives at least partial control of seedling blight initiated by infected seed, but poor or no control of seedling blight originated by soil-borne inoculum.

New pathogenic races of Cercospora oryzae affecting rice. RYKER, T. C. The control of *Cercospora* leaf spot, the most serious disease of rice in Louisiana, has been sought through the breeding of resistant strains. Of the mid-season and late varieties, Blue Rose has been classified as susceptible; and Rexoro, Fortuna, and Nira as resistant. Race 4, affecting only Fortuna, was reported in 1940. This race never built up to any appreciable degree. However, in 1945 several diseased fields of Fortuna were observed and a still greater number were observed in 1946. Isolates, when tested on the host differentials, indicated that a new race (Race 7) was involved, since, in addition to Fortuna, Blue Rose also was susceptible. Rexoro has become badly diseased, since 1944, with a widespread epiphytotic of Race 6. Nira, the only remaining resistant variety, showed some infection in 1945 and several severely infested fields were observed in 1946. Isolates, when tested,

showed that a new race had appeared and it is designated as Race 8. Of the 6 differential varieties used, Nira and Fortuna were susceptible, while Blue Rose, Blue Rose 41, Caloro, and Rexoro were resistant.

Source of seed potatoes and varied dosage of disinfectant in relation to control of seed-borne scab. SAMSON, R. W. Disinfected portions of 10 different lots of seed potatoes from mineral soils produced no greater percentages of scab-free and marketable potatoes than did nontreated portions of the same lots, when planted in Indiana muck soil. In direct comparison, treated portions of 15 different lots of seed potatoes originating from Indiana muck soils consistently produced higher percentages of scab-free and marketable potatoes than the corresponding nontreated portions, with wide differences among seed lots. Disinfection of the muck-grown seed potatoes apparently was not complete, for they yielded lower percentages of scab-free and marketable tubers than did either the treated or nontreated mineral soil stocks. Light and heavy dosages of sulphur applied to slightly scabby tubers of the variety Sequoia from Indiana muck resulted in yields of 42 and 77 per cent, respectively, of scab-free potatoes when grown in mineral soil. The nontreated check produced only 11 per cent scab-free tubers. Variable responses to treatment for seed borne scab may be due to variations in scab inoculum on different lots of seed potatoes.

The influence of climate on four leaf parasites of Zea mays in Guatemala. SEMENIUK, G., and J. R. WALLIN. Corns from the lowlands, highlands, and mountainous regions of Guatemala were planted at 6 locations in Guatemala, from sea level to 8200 ft. altitude. The climate at these locations ranged from tropical to temperate, rainfall from 40 to 150 inches, and corn maturity from 3 to 9 months. Corn rust (*Puccinia sorghi*) was the most prevalent severe disease at Tiquisate (100 ft.), Barcena (5000 ft.), Antigua (4900 ft.) and Quezaltenango (8200 ft.). *Helminthosporium turcicum* leaf blight was most prevalent and severe at Coban (4200 ft.) and Barcena. *Angiospora zeae* was prevalent in moderate amounts at Chocola (3200 ft.) and in trace amounts at Coban, Barcena, and Antigua. *Phyllachora zeae* occurred primarily on the earlier maturing sorts in slight amounts at Chocola and Coban and as traces at Barcena. The corns from the mountain regions maintained their resistance to corn rust at Quezaltenango, Barcena, and Antigua but failed at Tiquisate. Late highland corns were heavily rusted at Tiquisate, moderately at Quezaltenango, slightly at Barcena and Antigua. The early-maturing lowland corns were severely rusted only at Quezaltenango, Antigua, and Tiquisate, and moderately at Barcena. Climate influenced the prevalence and destructiveness of the leaf parasites in Guatemala. Selected locations served to uncover resistance. Two corns proved highly resistant to *Helminthosporium turcicum*. (Iowa State College and Guatemala Tropical Research Center.)

Root necrosis resistance in maize. SEMENIUK, G., J. R. WALLIN, and I. E. MELHUS. Collections of United States, Mexican, Guatemalan, and South American corns were tested for their reaction to *Pythium graminicola*, a destructive parasite of corn roots in Iowa. Altogether, 988 collections were included in 23 different trials. A standard procedure, developed from preliminary studies, was followed. In each test 40 different lots were planted in quadruplicate in steamed infested soil and in duplicate in steamed noninfested soil. Two inbred lines, R 4 and Hy, of known reaction to *P. graminicola*, were included in each trial. Twenty-one days after planting, the seedlings were removed and the soil washed from the roots. The severity of the pathogen on the seedlings was recorded in five classes based on the amount of root necrosis and root development and reduction in seedling height. Only six lots of corn, including 3 Guatemalan, 1 United States, and 2 Mexican collections, were in the most resistant class. Seventy-one lots, including 55 Guatemalan, 9 United States, 4 Mexican, and 3 United States-Guatemalan single-cross corns were in the next highest class. The majority of the corns were in the markedly susceptible classes 3, 4, and 5.

Plant-disease relation and classification of Fusaria. SHERBAKOFF, C. D. Many Fusaria are pathogenic and still more are commonly associated with various diseases. They exhibit a great variety of forms and occur universally on all sorts of substrata. Thus plant pathologists are often concerned in their identity and would welcome an adequate classification. The author's studies support Wollenweber's classification, with these reservations: Parasitic Fusaria to retain their original binomials, such as *F. lycopersici*, and the "form" to be used only for physiologic differences. Other Fusaria should be identified by their macroconidia in optimal development, together with characters of microconidia, conidiophores, chlamydospores, and others. No single character alone could hold throughout in any classification, and no overlapping of its extremes makes a character invalid. Observing these rules no one could possibly contrive such name as *F. roseum f.*

cerealis Snyder and Hansen. The great variability of Fusaria is more imaginative than real, although, like other organisms, they are affected greatly by environment. It must be kept in mind, however, that the value of the differences between some of the Fusaria may be on a par with that between varieties of roses or wheat. Knowledge of how to obtain optimal sporulation, and good photographs, will greatly improve the classification.

New methods of artificial seed inoculation for testing the resistance of spring and winter barleys against stripe, Helminthosporium gramineum. TAPKE, V. F. The writer has recently described the "mycelial-fragments" method of inoculating barley seed with the stripe fungus, *Helminthosporium gramineum.* The method has proved consistently effective and practical to apply. In further study it has been found that after the seed has been inoculated with mycelial fragments in aqueous suspension, the duration of incubating the seed at 7°–10° C. plays a critical rôle. With increase in length of incubation the severity of stripe increases but the percentage of seed germination decreases. This decrease is due partly to the fungus and partly to the increasing injury associated with the drying of seed that has developed longer sprouts in the longer periods of incubation. In general an incubation of 4 to 6 days seems best for a heavy stripe attack with only a small to moderate reduction in seedling emergence. In studies on the stripe resistance of winter barleys grown in the greenhouse a modification of the mycelial-fragments method plus seed vernalization has given both excellent infection and heading of the winter barley plants.

Cumulative benefits from the control of leaf spot of sour cherry in West Virginia. TAYLOR, CARLTON F. A cumulative spray experiment, started in 1939 on 2-year Montmorency cherry trees, has been continued through 1946. Over this period control of leaf spot (*Coccomyces hiemalis*) with Copper Hydro 40 or with Bordeaux mixture has been much superior to that obtained with all-season lime-sulphur schedules. In 1945 heavy early defoliation on the lime-sulphur-sprayed plots had stimulated the production of secondary growth on 64 per cent of the terminals by July 21. These secondary leaves were soon lost due to leaf spot, and some tertiary growth developed. Following this poor control of leaf spot an estimated 72 per cent of the branches on the lime-sulphur plots were killed by winter injury. Four applications of either Bordeaux or Copper Hydro afforded sufficient protection to avoid "second growth" and no winter injury ensued. Fruit yields in 1946 were 0.25, 28.3, and 43.0 lb. per tree, respectively, on the lime-sulphur, Bordeaux, and Copper Hydro series. Bordeaux-sprayed fruits were smaller than those sprayed with Copper Hydro (138.8 vs. 121.8 fruits per lb.). Trunk diameter increases have been significantly greater following copper sprays than following lime-sulphur.

Changes in pectin produced by isolates of Sclerotinia spp. TELLER, M. N., and E. O. MADER. Three isolates of *Sclerotinia spp.*, which were weakly, moderately, or strongly virulent as judged by the rate at which they rotted apples, were cultured on 1 per cent apple pectin solution. Viscosity, pH, and total acidity were determined at 24-hour intervals for 5 consecutive days. Noninoculated 1 per cent pectin was used as control. Though the pH of the medium was not changed by the isolates, remaining between 3.4–3.6, the total acidity was increased gradually by the weakly and moderately virulent isolates, and increased rapidly by the strongly virulent isolate. The initial relative viscosity of 3.18 (distilled water $=1$) was raised to 5.92 by the weakly virulent pathogen, and to 4.70 by the moderately virulent pathogen. The strongly virulent pathogen, however, lowered the relative viscosity of the medium to 1.41. The relative viscosity of the noninoculated control fluctuated very slightly. Similar viscosity values were obtained with the same isolates when this experiment was repeated six months later. There is an apparent correlation between the virulence of the fungus on apples and its effect on pectin.

Streptomycin production by Streptomyces griseus from peanut and soybean protein waste liquor. THORNBERRY, H. H. Peanut and soybean oil extracted meal fractions [(1) meal, (2) solution of protein and waste at pH 8.0, (3) protein precipitated at pH 4.5, and (4) waste liquor from protein precipitation] in varied amounts were added to a basal medium consisting of glucose 10 g., Bacto peptone 5 g., sodium chloride 5 g., distilled water 1000 ml., and H-ion concentration at pH 7.0. Production of streptomycin was by submerged culture in triplicate flasks shaken on a 2.5-inch reciprocating stroke at 100 cycles per minute and 28° C. for 3 days. Assays were by the paper-disc method. Peanut fractions 1, 2, and 3 were poor nutrients while fraction 4 at amounts equivalent to 30, 40, and 50 grams of the meal per liter gave yields as good as or better than yields with corn steep, about 100 units per ml. Soybean fraction 8 was a poor nutrient while fractions 1 at 1, 5, and 10 grams; 2 at 50 grams; and 4 at 20 and 30 grams per liter gave

production as good as or better than that with corn steep. These protein waste liquors are suitable nutrients for the production of streptomycin by submerged culture.

Preliminary studies on the nature of aversion in cultures of Diplodia zeae. ULLSTRUP, A. J. When two averting cultures of *Diplodia zeae* were grown together on potato-dextrose broth or on a synthetic medium with technical maltose as the source of carbon, and ammonium sulphate as the source of nitrogen, the filtrate of the medium, after sterilization, was toxic to either member of the averting pair, to any other culture of *D. zeae*, and to certain other species of fungi. Toxicity was evident in filtrates 6 days after seeding, and within 10–12 days after seeding filtrates were completely inhibitory to growth of *D. zeae* and a number of other fungi. When either member of an averting pair was grown singly, or a pair of compatible cultures grown together, in the media mentioned, no toxicity was expressed by the filtrate. The mycelial mat of a pair of averting cultures gained little in dry weight after 8 days of incubation, whereas the mycelial mat of a single culture or a pair of compatible cultures steadily increased in dry weight up to 18 days. The toxic material in the filtrate was stable to autoclaving for 20 minutes at 15 lb. pressure. When toxic filtrates were extracted with chloroform the fungistatic material was removed by the solvent, leaving the aqueous fraction nontoxic. Extraction residues from filtrates on which single or paired compatible cultures had grown were nontoxic. (U. S. Department of Agriculture and Purdue University Agricultural Experiment Station.)

Meadow nematodes from brown root rot of tobacco. VALLEAU, W. D., and E. M. JOHNSON. Roots of several varieties of burley tobacco in the brown-root-rot plot at Lexington, Kentucky, and in 132 other tobacco plots in the same series in which tobacco makes slow growth after setting, were found heavily invaded with what apparently is the meadow nematode, *Pratylenchus pratensis*. The same nematode was present in roots of several grasses, legumes, and weeds that precede tobacco in the rotations in 117 plots. Tobacco roots from a continuous tobacco–small grain cover crop rotation and from a virgin bluegrass sod plot, in both of which tobacco (black-root-rot resistant) starts quickly, have proved, so far, free from meadow nematodes. Five tobacco varieties, 2 susceptible and 3 having some resistance to brown root rot, reacted similarly to brown root rot at Harrow, Ontario, and at Lexington, Kentucky, suggesting that the disease at both stations is caused by the same agent. The injury caused by meadow nematodes to the small roots of tobacco would seem to be sufficient to account for brown-root-rot injury.

Seed treatments for fall- and winter-sown onions. VAUGHAN, EDWARD K., and W. D. MOORE. Various fungicidal dusts have been studied as seed treatments to prevent seed decay and pre-emergence damping-off in fall- and winter-sown onions in Georgia. The materials used throughout the experiments were Arasan (tetramethyl-thiuram-disulphide), zinc oxide, Fermate (ferric dimethyldithiocarbamate), and Semesan (hydroxymercuri-chlorophenol). Cuprocide (red cuprous oxide) and Spergon (tetrachloro-parabenzo-quinone) were used in the earlier tests, but results did not warrant their continued use. Arasan (1.5 per cent by weight) gave consistently good control throughout the tests and in 3 instances was significantly superior to any other material used. Fermate (1.5 per cent by weight) closely approached Arasan in effectiveness. Zinc oxide (2.0 per cent by weight), while fairly effective, did not give as consistently good results as Arasan and Fermate. Semesan was not effective. The effectiveness of these materials was not greatly influenced by fluctuations of soil temperature and soil moisture, or by soil type. Both Arasan and Fermate can be used over wide dilution ranges without loss of effectiveness or injury to the young seedlings.

A comparison of certain potato sprays in different localities in West Virginia. VAUGHN, J. R., and J. G. LEACH. A total of 12 different fungicides with and without DDT were tested, some in 3 localities under different environmental conditions. Among those giving best results were Bordeaux mixture, fixed copper, Dithane (disodium ethylene bisdithiocarbamate), and Dithane reaction product (Ile 178e, zinc ethylene bisdithio-carbamate). When used with DDT in 4- or 5-row replicated plots 60 feet long, Dithane was equal to Bordeaux, but when used on a commercial scale in one locality where late blight started early and was unusually severe, it was inferior to Bordeaux. Dithane reaction product in 1946 in small plots was better than Dithane and easier to use. Fixed copper with DDT gave slightly higher yields than any other spray tested except three exploratory chromate compounds. These were equal to fixed copper with DDT in the 1946 test under severe late blight conditions.

The use of the seedling inoculation technique for testing tomatoes for resistance to Verticillium wilt. VIRGIN, W. J., and J. O. MALOIT. The technique of seedling inocula-

tion as employed in determining resistance in tomato to *Fusarium* wilt can be applied with certain modifications in studying the resistance of tomato to *Verticillium* wilt. Tomato seedlings 3 inches high were inoculated by dipping the roots in a liquid suspension of *Verticillium albo-atrum*. To prepare the inoculum, mats of the fungus, grown on liquid media, were mixed in a Waring Blendor with water plus enough plain agar to make a thick consistency. Immediately after dipping, the seedlings were transplanted to sterile sandy loam soil in the greenhouse. The soil and air temperature was held as near 21° C. as possible throughout the test. Eight days after inoculation, wilting and yellowing of the primary leaves occurred, followed by yellowing in the secondary leaves. Internal thread-like strands of brown to black discoloration were observed in affected plants. Some of the plants died within 3 weeks after inoculation, while others having the described symptoms remained alive but were severely stunted. The organism was isolated from the stem and leaves of infected plants. Plants with no internal or external symptoms were considered tolerant or resistant.

Some phytopathological problems in Japan. WALDEE, E. L. Despite handicaps imposed by war and a feudalistic totalitarian system, Japanese phytopathologists have carried on their work with admirable success. Considerable progress has been made on the control of rice blast through resistant varieties and fungicidal sprays. Rice spraying is greatly aided by a rather effective epiphytotic forecasting service. The use of fungicidal sprays on cereals has been promoted by means of Government subsidies. Greater emphasis is now being placed on such locally destructive rice diseases as sclerotiosis, stem rot, and seedling blight. Control of the cereal rusts by resistant varieties is promising for wheat. Barley breeding is not so far advanced. Of special interest is wheat stripe (*Cephalosporium gramineum* Nishikado et Ikata) and barley bunt (*Tilletia pancicii* B. et R.). An elaborate research program on snow blight of cereals (*Typhula spp., Fusarium spp.,* and *Pythium spp.*) promises to increase greatly wheat and barley production throughout the heavy snow area. The excellent research on black rot of sweet potato has received official commendation. The principal causes of low yields of Irish potatoes are virus diseases. The seed-potato certification program has not proved effective, chiefly because of inadequate enforcement of disease tolerances. The Japanese plant-disease survey is unsatisfactory and is now being reorganized.

Improvement of cabbage for disease resistance. WALKER, J. C., and GLENN S. POUND. Selection for mosaic resistance has been continued within the yellows-resistant varieties, Wisconsin All Seasons, Wisconsin Ballhead, and Wisconsin Hollander. An improved strain of Wisconsin Ballhead, released in 1946 as Improved Wisconsin Ballhead, is distinctly higher in mosaic resistance. An improved strain of Wisconsin All Seasons ready for release is distinctly higher in mosaic resistance and more uniform in desired horticultural characteristics. Selection is being continued within this variety for still greater mosaic resistance and higher ascorbic acid content. Wisconsin Hollander contains only Type B resistance to yellows and is very susceptible to mosaic. By crossing with Wisconsin Ballhead and selecting for Type A yellows resistance and mosaic resistance, homozygous Type A yellows-resistant lines distinctly higher in mosaic resistance have been secured. Selection for horticultural type is still in process.

Natural and cultural occurrence of the ascogenous stage of Diaporthe phaseolorum var. sojae. WELCH, A. W. The ascogenous stage of *Diaporthe phaseolorum* var. *sojae* has been reported only from culture. It occurs naturally during late spring, under Iowa conditions, on soybean stems overwintered in the field. Perithecia are produced singly or in caespitose groups of 1 to 7 per stroma. The perithecial beaks vary from 280 to 546 μ in length; asci 27.2–40.8 × 6.8–8.5 μ; and ascospores 8.5–10.2 × 3.4–5.1 μ. Isolates obtained from the Phomopsis stage failed to produce perithecia in culture. Isolates obtained from the stem lesions of wilting and dying plants produced perithecia in approximately 30 days on potato-dextrose agar. In culture, 1 to 32 perithecial beaks have been observed per stroma. These beaks varied from 268 to 569 μ in length; asci 33.3–51.8 × 5.6–9.0 μ; and ascospores 9.3–11.3 × 2.5–4.6 μ. Stems with lesions collected during August and September produced perithecial beaks after 4–15 days in moist chambers. Pycnidia were produced abundantly on stems of maturing plants. Pycnidia and perithecia have not been observed within the same lesion. (U. S. Department of Agriculture and Iowa Agricultural Experiment Station.)

A method of correcting for soil variation in field tests. WELLMAN, R. H., H. W. THURSTON, JR., and F. R. WHALEY. It is often impossible to distinguish between treatments because of soil variations within replicates. Statistical designs, now known to compensate for this variation, are inflexible as regards numbers of treatments and replicates. Our method has no such restrictions. Consider an experiment where plots run

north and south and are arranged side by side. Thus the field runs east and west. Yield is recorded independently for north and south halves of each plot. Long narrow plots should be further divided. Only north half plot yields are now considered. Field average plot yield ($\overline{Y}$) and average plot yield per treatment (y) is determined. R is actual yield per plot. $\overline{Y}/y \times R = G$. G values include individual variation (error) and soil variation but exclude treatment effect. Plot G values in field order. Construct a smooth curve through them by customary procedures. Subtract $\overline{Y}$ from the smooth curve at the location of each plot. The resultant value is c. $\overline{Y}/(\overline{Y}+c) \times R = $ adjusted yield. Repeat for south half. Add adjusted yields for both halves. Resultant adjusted entire plot yields are subject to analysis. In a potato experiment, the uncorrected error variance was 2840; with triple lattice correction, 2129; with above correction, 1302. (Carbide and Carbon Chemicals Corp., Pennsylvania State College, and Linde Air Products.)

The development of increased tolerance to sodium arsenite by Sclerotium rolfsii and Sclerotium delphinii. WILSON, COYT. A strain of *Sclerotium rolfsii*, 1 of *S. delphinii*, and 1 intermediate between the 2 species have increased their tolerance for sodium arsenite from 125 p.p.m. to 150 p.p.m. during 5 transfer generations covering a period of approximately 10 months. Cultures that have been on arsenical media for 5 transfer generations grow approximately twice as fast on potato-dextrose agar containing 125 p.p.m. sodium arsenite as those that have been on media containing no arsenic. No sclerotia are formed on the arsenical media, and the mycelium is brown and appressed rather than white and fluffy. When cultures that have been grown on arsenical media are returned to potato-dextrose agar they form sclerotia that are indistinguishable from those formed in cultures grown continuously on arsenic-free potato-dextrose agar. Sectoring on arsenical media is rare. Occasionally a mutant arises that does not form sclerotia.

A survey of the fungi associated with peg and seed rots of peanuts in southern Alabama. WILSON, COYT. Isolations from pegs, shells, and seeds have revealed that the young peanut is often invaded by soil-borne fungi soon after it penetrates the soil. Infection is facilitated by, but is not dependent upon, various types of insect injury. Such miscellaneous fungi as *Penicillium, Aspergillus, Trichoderma, Rhizopus,* and other Mucors have comprised more than half of the cultures obtained. Of the remainder, *Sclerotium rolfsii, Sclerotium bataticola, Diplodia theobromae, Rhizoctonia spp.,* and *Fusarium spp.* were obtained in approximately equal proportions, although in any given sample any one of these may predominate. The results indicate that *Sclerotium rolfsii* may have been overemphasized in the past as a cause of peg rot and that the disease may be caused by any of several fungi. After the peanuts are harvested, another type of seed decay, concealed damage, becomes important. In the early stages of the disease, *Diplodia theobromae* has comprised more than 80 per cent of the fungi isolated. As the decay progresses, species of *Fusarium* and other secondary invaders are obtained more frequently.

Therapeutic treatments for bean rust. YARWOOD, C. E. Destruction of bean rust (*Uromyces phaseoli typica*) uredial pustules after they were visible to the naked eye at 4 days and more after inoculation has been accomplished with little host injury by treatment with cyanide gas, hydrogen sulphide gas, vapors from dilute lime-sulphur, penetrating lime-sulphur sprays, hot water, and hot air. With lime-sulphur sprays, the addition of a spreader, treatment of the plants during the morning, the water-soaking of the leaves with sprays of high-impact pressures, and the incubation of the treated plants in dark moist chambers until after natural darkness, all favored eradication, and the optimum concentration was about 0.3 per cent lime-sulphur. Several other sprays were relatively ineffective. With hot water the time for complete killing of rust pustules without leaf-killing ranged from about 80 min. at 35° C. to 2 sec. at 55° C., with a temperature coefficient of about 50. The killing of 4- to 6-day-old pustules usually left only faint chlorotic spots on the leaf, but killing of 8- to 10-day-old pustules left necrotic areas extending roughly to the limits of the rust mycelium.

Relation of soil moisture and nutrient concentration to the development of bean powdery mildew. YARWOOD, C. E. Pinto beans, grown at low soil moisture, supported a more luxuriant growth of mildew (*Erysiphe polygoni*) with more spores formed per unit area, less leaf necrosis, and with greater reduction in host yield, than at high soil moisture. The more vigorous mildew development on slow-growing plants at low soil moisture than on fast-growing plants at high soil moisture is a marked exception to the general rule that obligate parasites grow best on vigorous growing plants. On beans grown in water cultures containing ⅛, ¼, 1, 2, and 4 times the concentration of salts in standard Hoagland solution mildew development increased, especially on the primary leaves, with increasing nutrient concentration, while plant growth was best at the standard concentration. It is believed that similarities between low moisture in soil cultures and

high nutrient concentration in water cultures may explain the similarity in mildew response in these unfavorable environments for host growth, and that both situations lend further support to the idea of the xerophytic nature of many powdery mildews.

Greasy pod—a new virus disease of beans. ZAUMEYER, W. J., and H. REX THOMAS. In 1944 an apparently new mosaic was noted in much of the garden bean seed acreage of southern Idaho. In 1945 it was observed in Montana, Wyoming, and Colorado but was not so widespread in these States as in Idaho. The symptoms consist of a chlorosis and bronzing of the leaves and a shiny or greasy appearance of the pods, which are completely without pubescence and darker than normal ones. Unlike the symptoms of bean virus 1, the leaves of plants infected with greasy pod are thicker than normal with no puckering, malformation, or distortion, but are decidedly rugose. The plants are only slightly stunted, although the pods are frequently malformed because of improper ovule development. The most susceptible varieties are those that are tolerant only to bean virus 1; those resistant to greasy pod are resistant also to virus 1. In this respect greasy pod virus differs from the virus reported from New York and Idaho, which is highly infectious to some of the varieties definitely resistant to bean virus 1. The virus of greasy pod is seed-borne to about the same extent as bean virus 1. In the greenhouse the symptoms are similar to those produced by virus 1. Because of certain similarities in seed transmission, varietal resistance, and properties, greasy pod is believed to be a strain of bean virus 1.

Phytophthora cinnamomi in relation to avocado decline. ZENTMYER, GEORGE A., and L. J. KLOTZ. Evidence indicates that *Phytophthora cinnamomi* may be a primary factor in "decline" of avocado trees. This fungus is intimately associated with the disease that has killed several thousand large trees. Injury initially appears on poorly drained, wet soils. The most common underground symptom is destruction of feeder roots. The fungus has been shown capable of destroying small roots, causing cankers on larger roots and on the rootstock and trunk, even under nonwaterlogged conditions. The sudden "collapse" of avocado trees under the anaerobic conditions prevailing when excess water persists in the root zone may be entirely an oxygen relation, distinct from the slower root rot. Seedlings will "collapse" from waterlogging periods of 8 to 10 days. Seedlings in soil inoculated with *P. cinnamomi* will "decline" after two-day waterlogging periods. Treating soil from decline areas with steam or chloropicrin in the greenhouse has resulted in marked response of avocado seedlings over their growth in nontreated soil, further indicating the importance of a biological factor. Ethylene dibromide, Dowfume N (mixture of dichloropropane and dichloropropylene), chloropicrin, and 8-hydroxyquinoline benzoate, at low concentrations (0.01 per cent or less by volume) inhibit the growth of *P. cinnamomi* in soil in the laboratory. Such concentrations of ethylene dibromide and 8-hydroxyquinoline benzoate are not toxic to avocado roots.

ELECTRON MICROSCOPE STUDIES ON TOBACCO–MOSAIC VIRUS

THORBJORN SIGURGEIRSSON[1] AND W. M. STANLEY

INTRODUCTION

In the original description of purified tobacco-mosaic virus it was reported that, on the basis of results obtained in two preliminary experiments on osmotic pressure and diffusion, the purified material appeared to possess a molecular weight of the order of a few millions (26). Since then numerous publications on the size and shape of tobacco-mosaic virus have appeared (5–21, 23, 25, 27, 28, 30). It was early recognized that the original method of preparation resulted in aggregation of the virus and since 1936 the virus has been purified in this laboratory by differential centrifugation, for this method causes little or no aggregation (1, 23, 24). Before the advent of the electron microscope it was estimated by indirect methods, based on sedimentation, diffusion, viscosity, and X-ray data, that the virus particle possessed a molecular weight of about 40 millions and was an elongated particle about 12 to 15 mμ in diameter and about 400 mμ in length (5, 16, 17). In an early study with the electron microscope the predominating unit present in centrifugally purified virus preparations was found to be a rod about 15 mμ in diameter and about 280 mμ in length (28). Similar results were obtained in further, more precise, studies involving indirect methods (20). However, in the electron micrographs, images of particles formed by the end-to-end aggregation of this unit, as well as of particles having shorter although variable lengths, were also found to be present. Lauffer (19) has shown that the sedimentation constant of the infectious principle of tobacco-mosaic virus is the same, within a probable error of 6 per cent, as that of the rod 15 by 280 mμ. His results prove that, in the case of the present material, the sole carriers of virus activity cannot possibly be particles any smaller than half the size of the predominating particles. This result is in complete accord with much earlier work from this laboratory relating virus activity directly to the nucleoprotein of high molecular weight. In the absence of data to the contrary is has seemed justifiable to conclude that tobacco-mosaic virus is actually a particle 15 by 280 mμ. It is recognized that, as with any biologically active material, the biological activity may at some future time be found to be associated with smaller particles. Perhaps with this in mind, Bawden and Pirie have, from time to time, advanced the idea that the rods 15 by 280 mμ are really aggregates and that the true virus is a much smaller, perhaps even spherical, particle (1, 2). In the absence of supporting experimental data they have been forced into the rather unusual position of "postulating that the primary virus particles are unable to exist free in solution" (3). Obviously if the virus particle cannot exist free in solution it is removed from the ordinary realm of experimentation and it becomes difficult, if not

impossible, to prove or disprove its real existence by means of experiments. In the absence of direct experimental proof for its existence such a particle must remain a figment of the imagination. In view of the large amount of experimental data which indicates that tobacco-mosaic virus activity is directly associated with the particles 15 by 280 mμ, it would appear desirable to continue to accept the 15 by 280 mμ particle as tobacco-mosaic virus until such a time as virus activity is proved experimentally to be associated with a smaller particle.

It was found in earlier work that the characteristic rods 15 by 280 mμ could be demonstrated by means of the electron microscope in the unpurified juice pressed directly from a tobacco-mosaic-diseased plant (27). However, because of the lack of contrast of the rods with respect to residual extraneous material, the micrographs were rather unsatisfactory. The new shadow-casting technique (29) provides a method for increasing the contrast and in the present investigation excellent micrographs of unpurified infectious juice have been obtained. These show the presence of large numbers of a unit 15 by 280 mμ. In addition, in the present investigation, the rods of shorter and variable lengths that are known to be present in centrifugally purified preparations of tobacco-mosaic virus have been separated by fractional centrifugation and studied by means of the electron microscope and by means of virus-activity measurements. The results obtained provide additional reasons for considering that the rods 15 by 280 mμ occur within the plant cells and that these are, in fact, tobacco-mosaic virus.

EXPERIMENTAL WORK

In the present investigation an RCA Console Model electron microscope, type EMC-1, was used. The accelerating voltage was 30,000 volts and the magnification yielded by the microscope was 5800. The specimens were mounted on thin collodion membranes supported by copper screens. The virus samples were first diluted to a suitable degree by the addition of distilled water. Purified preparations were usually used at a concentration of 10^{-4} gm. per cc. A small drop of the diluted solution was placed on the collodion membrane and allowed to dry. In most cases the shadow-casting technique (29), involving the evaporation of gold on the screen in a vacuum at an oblique angle to the surface of the membrane, was used. In order to permit more accurate measurements of particle lengths, the image on the plate was enlarged photographically six times to yield a total magnification of 35,000.

In the first experiment young Turkish tobacco plants grown in the greenhouse were infected with tobacco-mosaic virus. Twenty-five days later the plants were harvested and frozen overnight. The frozen plants were put through a meat grinder and the juice was pressed from the mash after thawing. Electron micrographs were made of this juice directly after preparation and after standing for 1 and 20 days at 4° C. Samples of the juice were diluted with 50 volumes of distilled water immediately before examina-

tion. Figure 1 shows an electron micrograph of the juice directly after preparation and figure 2 shows the size distribution of the rod-like particles present in several pictures of this sample. All rod-like particles within given areas were included in the measurements. The total number of particles measured was 1350. It is obvious from figures 1 and 2 that most of the particles are about 280 mμ in length but that there are a few shorter as well as longer rods. Most of the longer rods fall into two groups having lengths approximately 2 and 3 times the length of the predominating unit. The very small particles forming the background for the rods are not characteristic of juice from diseased plants, for they are present in micrographs of untreated
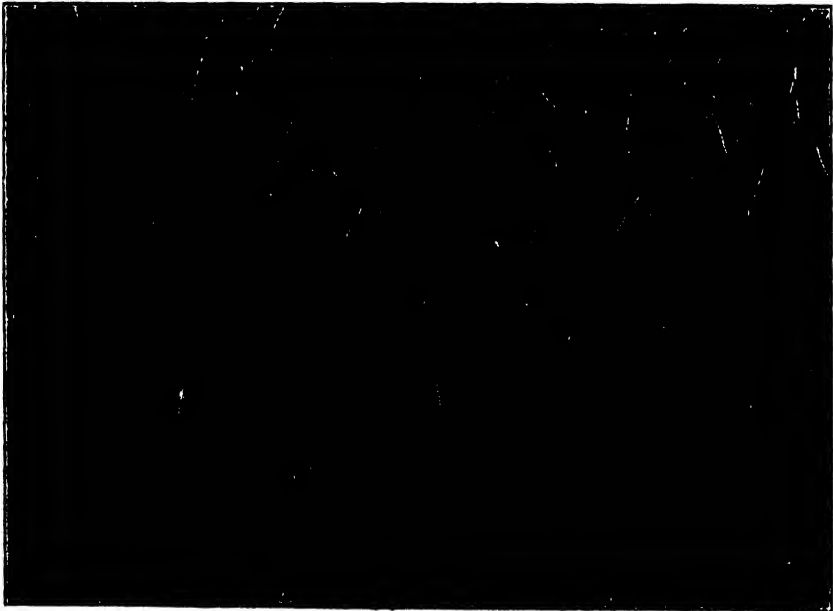

FIG. 1. Electron micrograph of the freshly expressed juice from tobacco-mosaic-diseased Turkish tobacco plants. Juice diluted with 50 volumes of distilled water and mount prepared with gold by the shadow-casting technique. The magnification is 23,200.

juice from normal Turkish tobacco plants. It is difficult to count accurately the particles of smallest size and for this reason the lower limit shown on the size distribution diagrams does not have any real significance.

Figure 3 shows the size distribution of rod-shaped particles present in micrographs taken of the juice after standing for 1 day at 4° C. It can be seen that most of the particles still fall in the size group near 280 mμ but that the group at double this size is much larger. Figure 4 shows an electron micrograph of the same juice after standing at 4° C. for 20 days. It is obvious that the proportion of rods of double length is much greater. It can be seen from the size distribution, which is shown in figure 5, that there are two major groups and that the group consisting of double length particles is even larger than the group having lengths near 280 mμ. There are, in ad-

figure 6 and in similar micrographs. The preparation contains a large group of particles having lengths near 280 mμ together with a large group having shorter although variable lengths. These shorter particles are usually not found in large amounts in micrographs of virus preparations obtained by centrifugation for one hour or less at 24,000 r.p.m. followed by solution of the pellets with gentle stirring and their presence in the present preparation may be due to the vigorous mechanical stirring to which the preparation was subjected. Considerable evidence has been obtained which indicates that
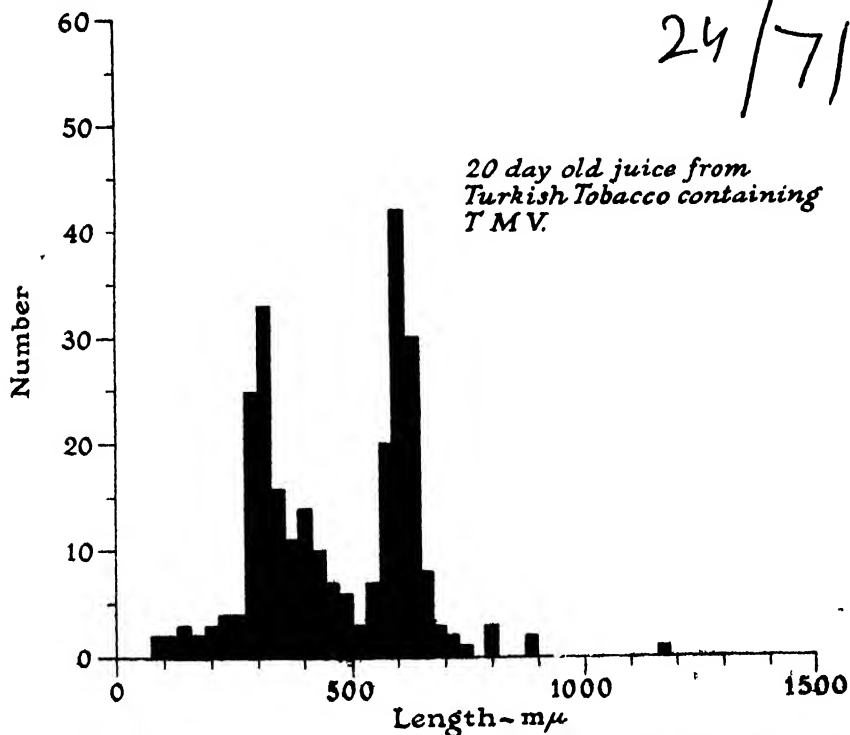

FIG. 5. Size distribution of rod-shaped particles present in the juice pressed from tobacco-mosaic-diseased Turkish tobacco plants after standing for 20 days at 4° C. Prepared from figure 4 and similar micrographs.

virus activity is associated with the rods 280 mμ in length (17, 19, 28). It seemed of interest therefore to secure an essentially pure preparation of the shorter rods so that it might be possible to determine directly their virus activity.

Accordingly, in a third experiment a comparable fairly concentrated preparation of virus was centrifuged for 2.5 hours at 24,000 r.p.m. The supernatant liquid was removed and centrifuged for 40 minutes at 24,000 r.p.m. The supernatant liquid from this centrifuge run, which was found to contain 0.15 mg. of protein per cc., was examined. It can be seen from the distribution of particle lengths shown in figure 8 that about 98 per cent of the particles are shorter than 280 mμ. The specific biological activity of this

preparation was compared with that of the original virus preparation by means of the half-leaf method on *Nicotiana glutinosa* plants (22). A solution containing 0.1 mg. of the short rods per cc. and a solution containing 0.001 mg. of the original virus preparation per cc. were used. The solution of short rods gave about 10 lesions per half-leaf, whereas the original virus preparation gave about 30 lesions per half-leaf. Since there was a hundredfold difference in concentration it is obvious that, at the same concentration, the virus activity of the preparation of short rods is less than 1 per cent that of the original virus preparation. This small residual activity is prob-
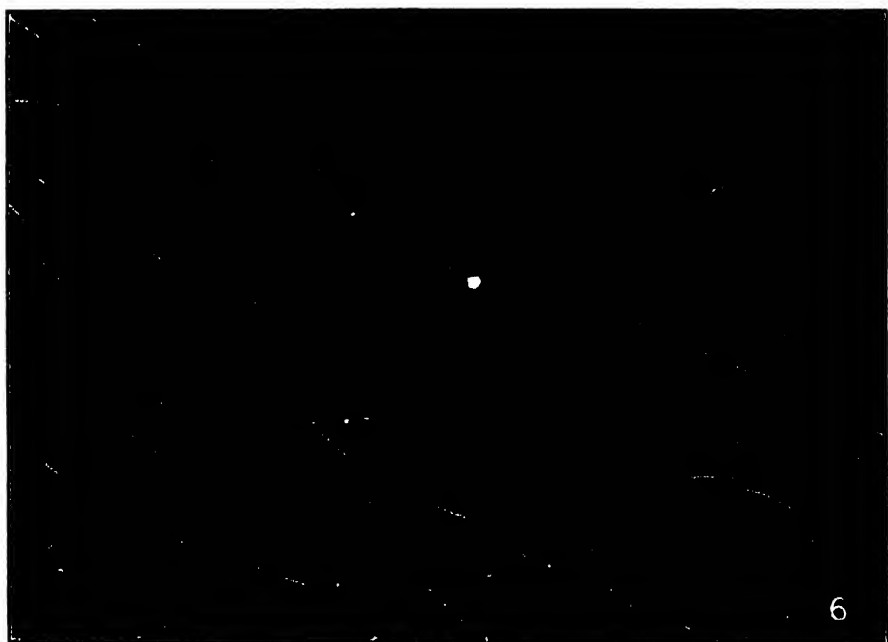

FIG. 6. Electron micrograph of tobacco-mosaic virus preparation obtained by centrifuging the freshly expressed clarified juice of tobacco-mosaic-diseased Turkish tobacco plants for 1 hour at 24,000 r.p.m. The preparation was diluted with distilled water to a concentration of 10^{-4} gm. of protein per cc. The mount was prepared with gold by the shadow-casting technique. The magnification is 23,200.

ably due to the few rods of 280 mμ length or longer still remaining in the preparation. The number of these rods is only 2 per cent of the total number of rod-like particles, but by weight this would account for about 10 per cent of the total protein content of the solution, hence a biological activity about 10 per cent of that of the original virus preparation would be expected. The fact that the activity was less than 1 per cent shows that the activity of the long rods is reduced, either intrinsically or possibly because of an inhibitory effect caused by the short particles. In any case it seems reasonable to conclude that the rods shorter than about 280 mμ are devoid of virus activity. The source of these short rods is not known with certainty. They seem to be present in the juice as it is pressed from the plant material. It is

possible that they are formed by the break-up of the virus rods due to thermal agitation.   In the case of centrifugally purified virus preparations the short rods may result from mechanical stresses during centrifugation and during the subsequent solution of the virus pellets by mechanical stirring.   That rods can be broken by mechanical stress is shown in figure 9, which is an electron micrograph of a mount of purified cucurbit-mosaic virus in which the collodion film has broken.   The stretching of this film has resulted in the breaking of several virus rods.
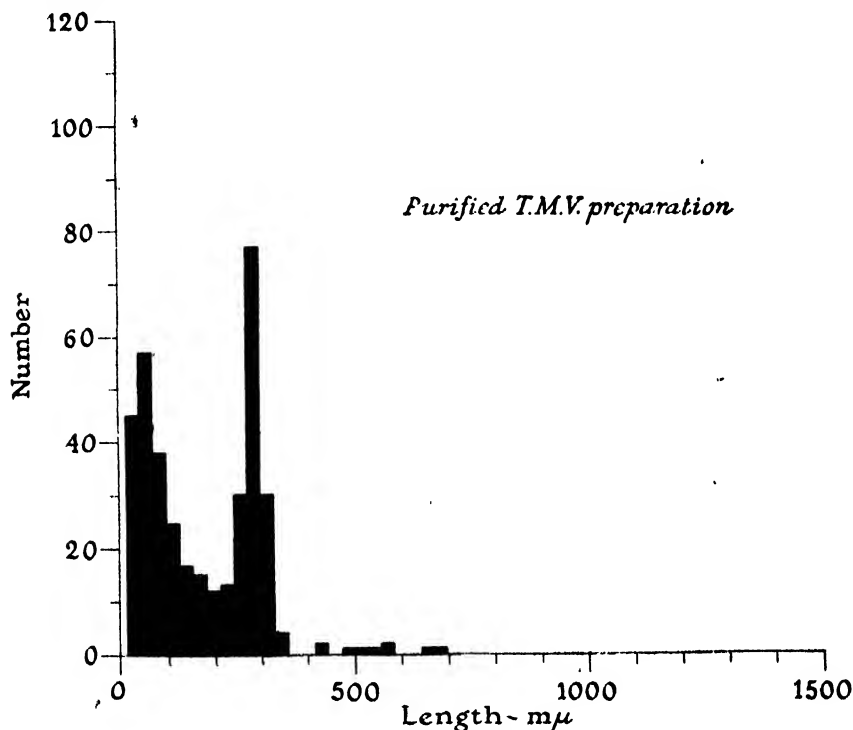

FIG. 7.   Size distribution of particles present in centrifugally purified tobacco-mosaic virus preparation.   Prepared from figure 6 and similar micrographs.

The cause of the end-to-end aggregation which occurs when infectious juice is allowed to stand is not known.   In a purified virus sample suspended in disodium phosphate buffer at pH 7 it does not seem to take place to any appreciable extent.   The juice has a pH of 5.4, but decreasing the hydrogen ion concentration by means of a buffer does not prevent the aggregation, for it takes place even at pH 9.   An attempt to break up the long rods by decreasing the hydrogen ion concentration was not successful.   At about pH 11.3 the virus particles are partially dissolved, as shown in figure 10, but no break-up into smaller parts seems to take place.   When the hydrogen ion concentration is adjusted around or below the isoelectric point (pH 3.5), there seems to be an increase in the rate of end-to-end aggregation, but another process, the side-by-side aggregation, appears to come into prominence.

Figure 11 shows a micrograph of a sample of fresh juice which has been brought to pH 2.2 by the addition of hydrochloric acid. This picture gives a good idea of the flexibility of the virus rods. Near the middle of the picture a bundle of rods is seen to bend sharply as it crosses over a single rod lying on the collodion membrane. This side-by-side aggregation seems to be identical with beginning crystallization and as the picture is sharp enough to show the individual rods, the distances across known numbers of these can be measured. These measurements give a value of about 150 Å for the diam-

Small sized fraction obtained
from centrifuged, purified
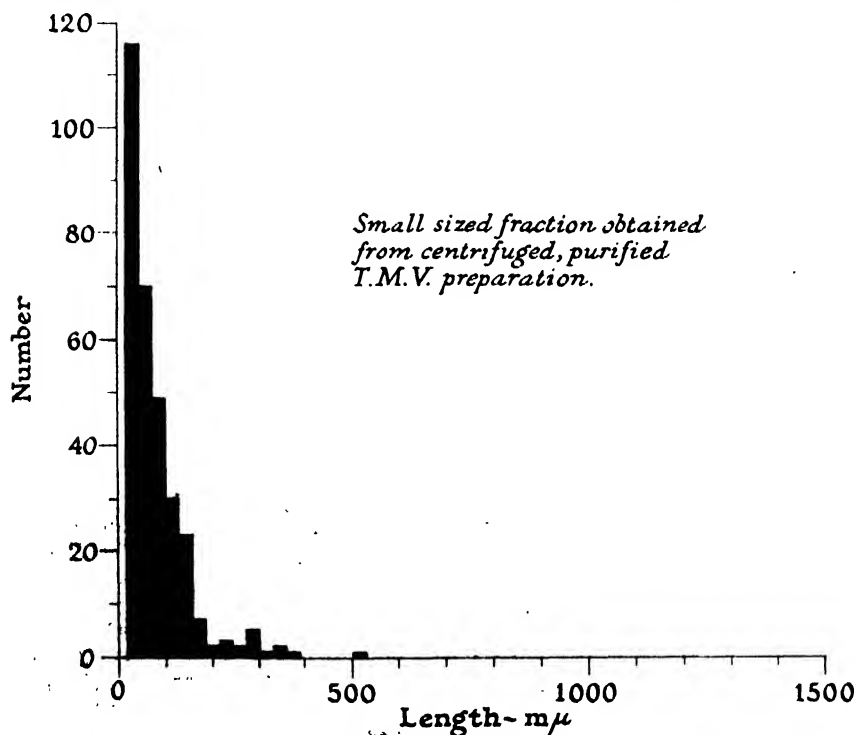T.M.V. preparation.

FIG. 8. Size distribution of rod-shaped particles present in preparation obtained by centrifugal fractionation of material similar to that described in figures 6 and 7.

eter of the individual rods. This value is in agreement with the value obtained by Bernal and Fankuchen (5) in X-ray studies.

DISCUSSION

The demonstration, not only that rods 15 by 280 mμ exist in the freshly expressed untreated juice of tobacco-mosaic-diseased Turkish tobacco plants, but especially that most of the rod-like particles present in such juice are essentially 15 by 280 mμ is considered of importance. If, as Bawden and Pirie (3) have postulated, the primary virus particle is small and not greatly elongated and unable to exist free in solution, it would appear most unusual for such small particles to aggregate, within the few minutes required to press out the juice, to form essentially uniform rods 15 by 280 mμ

FIG. 9.   Electron micrograph of cucurbit-mosaic virus particles that have been broken by the breaking and stretching of collodion membrane of mount.

FIG. 10.   Electron micrograph of tobacco-mosaic virus particles in infectious juice following exposure to pH 11.3.   Mount prepared with gold by the shadow-casting technique.   The magnification is 23,200.

in size.   The real existence of biologically active particles 15 by 280 mμ
has been amply demonstrated and it seems more logical to assume, as has
been done in this laboratory for some years, that these particles represent
tobacco-mosaic virus.   If one wishes to postulate the existence of biologically
active particles of smaller size, it should be accompanied by acceptance of
the burden of proof for the demonstration of the real existence of such
particles.   In the present investigation, as well as in work by Bawden and
Pirie, preparations of particles of smaller size have been obtained, but have
been found to possess little or no virus activity.   Despite the absence of
virus activity Bawden and Pirie persist in referring to such material as
"virus."   The essence of a virus is its virus activity and it seems unwise to

Fig. 11.   Electron micrograph of tobacco-mosaic virus particles in infectious juice
following adjustment to pH 2.2.   The mount was prepared with gold by the shadow-
casting technique.   The magnification is 23,200.

refer to a material possessing no virus activity as "virus."   Much experi-
mental evidence exists which indicates that the rods 15 by 280 mμ represent
the minimal biologically active units of tobacco-mosaic virus and it would
appear reasonable to continue to accept this material as tobacco-mosaic virus
until it can be proved experimentally that virus activity is associated with
units of smaller size.

The origin and properties of the particles smaller than 15 by 280 mμ and
the end-to-end aggregation of the 15 by 280 mμ units represent fields which
will require considerable additional investigation.   Little is known con-
cerning the origin of the rods shorter than 280 mμ, but there seems to be
general agreement that they may arise from the break-up of the longer rods
as a result of thermal or mechanical stresses.   There is some evidence which

indicates that the joint which is formed by the end-to-end union of two rods 280 mµ in length is just as strong mechanically as other points along the newly formed rod 560 mµ in length. Thus the end-to-end union of rods followed by the breaking up of the long rod into rods of uneven lengths could serve to explain the existence of rods of variable lengths, such as those shown in the micrographs. It has been demonstrated quite conclusively that preparations of the very short rods possess no virus activity, but the question of the virus activity of aggregates of the 15 by 280 mµ units is less clear, although there is some evidence that such aggregates possess a slightly diminished virus activity (23). It is possible that any rods that are about 280 mµ or more in length can serve as infective units, although multiples of the basic unit may be necessary. It does seem likely that the conversion of a preparation consisting essentially of rods about 280 mµ in length into a preparation consisting essentially of rods about 560 mµ in length should be accompanied by a 50 per cent reduction in specific virus activity due to the reduction in the actual number of particles present.

It will be difficult, if not impossible, to demonstrate conclusively that all of the tobacco-mosaic virus within the cells of diseased Turkish tobacco plants exists in the form of particles of a given uniform size. However, the present demonstration that most of the rod-shaped particles present in the freshly expressed juice of diseased plants are about 15 by 280 mµ in size, the isolation of most of the biologically active material in the expressed juice in the form of rods 15 by 280 mµ and Beale's work on the nature of virus within living cells (4), provide considerable justification for the assumption that tobacco-mosaic virus exists within plant cells in the form of rods 15 by 280 mµ.

SUMMARY

Electron micrographs, prepared by the shadow-casting technique, of the freshly expressed juice of tobacco-mosaic-diseased Turkish tobacco plants show that most of the rod-shaped particles present are 15 by 280 mµ in size. On standing at 4° C. many of these particles in the juice join end-to-end to form particles of greater length. At the end of one day a significant increase in the number of particles of double length was evident and at the end of 20 days the number of particles of double length was greater than the number of particles having lengths near 280 mµ. The joints formed by the end-to-end union of particles appeared to be as strong mechanically as other positions along the rods. Some of these elongated particles appear to be broken up by thermal or mechanical stresses to form particles of shorter and variable lengths. Particles less than about 280 mµ in length appear to possess no virus activity.

The results provide additional justification for the assumption that the basic infective unit of tobacco-mosaic virus is 15 by 280 mµ in size.

THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY,
    THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
        PRINCETON, NEW JERSEY.

LITERATURE CITED

1. BAWDEN, F. C., and N. W. PIRIE. The isolation and some properties of liquid crystalline substances from solanaceous plants infected with three strains of tobacco mosaic virus. Proc. Roy. Soc. London 123: 274–320. 1937.

2. ———————, and ——— ———. Plant viruses. I. Serological, chemical and physicochemical properties. Tabulae Biologicae 16: 355–371. 1938.

3. ——— ———, and ——— · ———. The separation and properties of tobacco mosaic virus in different states of aggregation. Brit. Jour. Exp. Path. 26: 294–312. 1945.

4. BEALE, HELEN P. Relation of Stanley's crystalline tobacco-virus protein to intracellular crystalline deposits. Contr. Boyce Thompson Inst. 8: 413–431. 1937.

5. BERNAL, J. D., and I. FANKUCHEN. X-ray and crystallographic studies of plant virus preparations. Jour. Gen. Physiol. 25: 111–165. 1941.

6. ERIKSSON-QUENSEL, I. B., and T. SVEDBERG. Sedimentation and electrophoresis of the tobacco-mosaic virus protein. Jour. Amer. Chem. Soc. 58: 1863–1867. 1936.

7. FRAMPTON, V. L. On the molecular weight of the tobacco-mosaic protein. Phytopath. 29: 495–497. 1939.

8. ——— ———. On the molecular weight of the tobacco mosaic virus protein. Science 90: 305–306. 1939.

9. ——— ———. On the size and shape of the tobacco mosaic virus protein particle. Science 95: 232–233. 1942.

10. ——— ———, and A. M. SAUM. An estimate of the maximum value for molecular weight of the tobacco mosaic virus protein. Science 89: 84–85. 1939.

11. GOWEN, J. W. The size of the tobacco mosaic particle from X-ray determinations. Proc. Nat. Acad. Sci., Washington 26: 8–10. 1940.

12. HILLS, CLAUDE H., and C. G. VINSON. Particle size of tobacco mosaic virus. Mo. Agr. Exp. Stat. Res. Bull. 286. 1938.

13. KAUSCHE, G. A. Ueber den Mechanismus der Goldsolreaktion beim Protein des Tabakmosaik und Kartoffel-X-virus. Biol. Zentr. 60: 179–199. 1940.

14. ——— ———, and H. RUSKA. Die Struktur der "kristallinen Aggregate" des Tabakmosaikvirusproteins. Biochem. Z. 303: 221–229. 1939.

15. ——— ———, and ——— ———. Die Sichtbarmachung der Adsorption von Metallkolloiden an Eiwisskörper. I. Die Reaktion Kolloides Gold. Kolloid-Zeitschr. 89: 21–26. 1939.

16. LAUFFER, M. A. The viscosity of tobacco mosaic virus protein solutions. Jour. Biol. Chem. 126: 443–453. 1938.

17. ——— ———. The molecular weight and shape of tobacco mosaic virus protein. Science 87: 469–470. 1938.

18. ——— ———. Ultracentrifugation studies on tobacco mosaic and bushy stunt viruses. Jour. Physic. Chem. 44: 1137–1146. 1940.

19. ——— ———. The sedimentation rate of the infectious principle of tobacco mosaic virus. Jour. Biol. Chem. 151: 627–634. 1943.

20. ——— ———. The size and shape of tobacco mosaic virus particles. Jour. Amer. Chem. Soc. 66: 1188–1194. 1944.

21. LEA, D. E. Determination of the sizes of viruses and genes by radiation methods. Nature 146: 137–138. 1940.

22. LORING, H. S. Accuracy in the measurement of the activity of tobacco mosaic virus protein. Jour. Biol. Chem. 121: 637–647. 1937.

23. ——— ———, M. A. LAUFFER, and W. M. STANLEY. Aggregation of purified tobacco mosaic virus. Nature 142: 841–842. 1938.

24. ——— ——— ———, and W. M. STANLEY. Isolation of crystalline tobacco mosaic virus protein from tomato plants. Jour. Biol. Chem. 117: 733–754. 1937.

25. MELCHERS, G., G. SCHRAMM, H. TRURNIT, and H. FRIEDRICH-FREKSA. Die biologische, chemische und elektronenmikroskopische Untersuchung eines Mosaikvirus aus Tomaten. Biol. Zentr. 60: 524–556. 1940.

26. STANLEY, W. M. Isolation of a crystalline protein possessing the properties of tobacco-mosaic virus. Science 81: 644–645. 1935.

27. ——— ———. Viruses and the electron microscope. Chronica Botanica 7: 291–294. 1943.

28. ——— ———, and T. F. ANDERSON. A study of purified viruses with the electron microscope. Jour. Biol. Chem. 139: 325–338. 1941.

29. WILLIAMS, R. C., and R. W. G. WYCKOFF. The thickness of electron microscopic objects. Jour. Appl. Physics 15: 712–716. 1944.

30. WYCKOFF, R. W. G. Molecular sedimentation constants of tobacco mosaic virus proteins extracted from plants at intervals after inoculation. Jour. Biol. Chem. 121: 219–224. 1937.

THE EFFECTIVENESS OF D-D AS A SOIL FUMIGANT IN HAWAII[1]

J . S . McFarlane and M . Matsuura

Numerous chemicals have been tested as soil fumigants in controlling the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, and other plant pathogens in the soil. Until recently the most successful and widely used material has been chloropicrin. The high cost and difficulties of applying this fumigant have precluded its large-scale field use with most vegetable and agronomic crops. A recently discovered fumigant known as D-D[2] is much easier to apply and cheaper to produce. The purpose of this study has been to determine the effectiveness of D-D as a seedbed and field fumigant under Hawaiian conditions. Although primary emphasis has been placed on nematode control, data are presented which indicate that it may be effective against other soil-borne organisms.

REVIEW OF LITERATURE

The effectiveness of D-D as a soil fumigant was first reported by Carter (1) in 1943. Carter's results were particularly striking in an area where a complex including at least *Anomala* beetle larvae (*A. orientalis*), nematodes, and pythiaceous fungi had resulted in serious plant failure. Pineapple plants in treated areas gradually showed increasing improvement over non-treated checks. Carter concluded that, apart from its immediate effect in reducing populations of harmful organisms, it had also affected beneficially the soil complex.

Christie (4) found D-D to have an effective killing range of from 9 to 12 inches when applied at a depth of 6 inches in a Berwyn loam soil. Variation in killing range from test to test did not seem to be correlated with soil moisture or temperature. D-D was more effective against nematodes in undecomposed roots than was chloropicrin.

Parris (5) found D-D to be an effective nematocide at rates as low as 150 lb. per acre in a silt-loam soil without a soil cover other than a wetting of the surface. He was able to demonstrate very little fungicidal value from studies with damping-off fungi, species of *Rhizoctonia, Fusarium.* and *Pythium*. A slight phytocidal action was noted if plants were set out too soon after treatment. A delay of at least 2 weeks between treatment and planting was recommended. The time interval required between treatment

and planting varied with the dosage and the plant used. D–D was found to be effective as a nematocide in a cold soil (30°–40° F.).

Tam (6) found D–D to be less effective in suppressing nitrification than was chloropicrin. Carter (2) found water solutions of D–D to be effective against the larvae of *Anomala orientalis* Waterh. and *Adoretus sinicus* Burm. in soil around the roots of actively growing nursery stock. Carter (3) reports that the 1–3 dichloropropene fraction of D–D is the most toxic but that there is synergism between this and other fractions.

<center>FIELD EXPERIMENTS</center>

<center>*1943*</center>

A comparison of D–D and chloropicrin as soil fumigants for vegetable crops was made at the Poamoho Experimental Farm, island of Oahu, in the fall of 1943. The study was a cooperative investigation between the University of Hawaii Agricultural Experiment Station and the Pineapple Research Institute. The comparison was made in a red lateritic soil in which a heavy population of the root-knot nematode had been built up by earlier crops of tomatoes, carrots, and beets. Four treatments, including the check, were randomized using 5 replications. Fumigants were injected September 1 and 2 at a depth of 5 to 6 inches with Vermorel injectors at staggered points 10.4 inches apart in rows 9 inches apart. The soil moisture content at depths of 2, 6, and 10 inches, respectively, was 27.0, 34.3, and 34.7 per cent at the time of application. The physical condition of the soil was poor and was not favorable for the most effective fumigation. No soil cover nor water seal was used.

The test crops used in the experiment were Morse's Bunching carrots and Victor tomatoes. Plantings were made September 10 on either side of an irrigation furrow which passed through the center of each plot. Carrot seed was planted on one side of the furrow and 20-day-old tomato seedlings transplanted on the opposite side. Fertilization and care were uniform, agreeing with the established practices at Poamoho.

TABLE 1.—*Comparison of the effect of soil fumigation with D–D and chloropicrin on the development of root knot and on the yield of carrots at Poamoho, Oahu, in 1943*

Treatment			Nematode infestation				Yield in pounds per plot	
			Percentage of plants in each class			Mean class value	Total	Marketable carrots
Material	Pounds per acre	No. of plants exam- ined	None 0	Slight 1	Moder- ate to severe 2			
Check	...	685	0.0	30.9	69.1	1.72	5.3	3.8
D–D	200	948	23.3	59.9	16.8	0.91	14.4	12.6
D–D	400	944	80.0	17.0	2.9	0.22	17.7	16.1
Chloropicrin	400	1135	75.4	18.8	5.8	0.29	19.0	17.5
Difference required for significance at 5 per cent level						0.30	7.7	7.3

TABLE 2.—*Comparison of the effect of soil fumigation with D-D and chloropicrin on the development of root knot and on the yield of tomatoes at Poamoho, Oahu, in 1943*

Treatment			Nematode infestation					Yield in pounds per plant
			Percentage of plants in each class				Mean class value	
Material	Pounds per acre	No. of plants exam- ined	None	Slight	Moder- ate	Severe		
			0	1	2	3		
Check		93	0.0	1.1	32.2	66.7	2.68	1.00
D-D	200	95	22.1	64.2	12.6	1.1	0.89	2.11
D-D .	400	69	55.1	39.1	1.5	4.3	0.61	2.23
Chloropicrin	400	102	51.0	42.2	3.9	2.9	0.56	1.94
Difference required for significance at 5 per cent level							0.41	0.67

Data on root-knot infestation of the tomatoes were obtained by removing alternate plants 26 days after transplanting. All green fruit was picked and weighed when the remaining plants were 11 weeks old. The carrots were harvested when 4 months old, examined for nematode infestation, graded, and weighed. Results are in tables 1 and 2.

Bacterial wilt, caused by *Bacterium solanacearum* E.F.S., destroyed 18.7 per cent of the tomato plants during the 11 weeks of the test. Fusarium wilt, caused by *Fusarium bulbigenum* var. *lycopersici* Woll. u. Reink., destroyed an additional 2.0 per cent of the plants. Data were obtained on the occurrence of these two diseases and are presented in table 3. A trend was observed for the incidence of Fusarium wilt to be less and that of bacterial wilt to be greater following fumigation with D-D. The incidence of both Fusarium wilt and bacterial wilt tended to be lower following fumigation with chloropicrin. The differences between treatments were not statistically significant, however.

The data from both the carrot and tomato tests show that D-D and chloropicrin materially reduced nematode injury. None of the treatments entirely eradicated nematodes, however. A few heavily infested plants were found in every plot. Comparable control of nematodes was obtained with D-D and chloropicrin at the rate of 400 lb. per acre. D-D at the rate of 200 lb. per acre markedly reduced nematode infestation but was not so effective as the 400 lb. rate in the carrot treatments.

Both the total and marketable yields of carrots were increased by the

TABLE 3.—*Influence of soil fumigation with D-D and chloropicrin on the occurrence of Fusarium wilt, Fusarium bulbigenum var. lycopersici, and bacterial wilt, Bacterium solanacearum, of tomatoes at Poamoho, Oahu, in 1943*

Treatment	Fusarium wilt	Bacterial wilt
	Per cent	*Per cent*
Check	4.3	18.0
D-D, 200 lb./acre	2.0	22.0
D-D, 400 lb./acre	0.6	29.2
Chloropicrin, 400 lb./acre	0.9	6.8

fumigation treatments. The lighter dosage of D–D produced striking gains which were not significantly exceeded by 400-lb. applications of either D–D or chloropicrin. The yields following application of 400 lb. of D–D and chloropicrin were similar. Yields of green tomato fruits were approximately doubled by the fumigants. There were no significant differences between the fumigated tomato plots.

1944

The 1944 experiments were conducted at the Poamoho Experimental Farm in an area similar to that used in 1943. A heavy infestation of nematodes had been built up in an earlier planting of tomatoes. Soil temperatures ranged from 65° F. to 80° F. during the course of the experiment. Lettuce was used as the test plant. Data were gathered on the rate, depth, and methods of D–D application.

Rate of application. The 1943 tests showed that D–D was as effective as chloropicrin in controlling the root-knot nematode, but more data were

TABLE 4.—*Influence of the rate of application of D–D on root-knot development and on the yield of lettuce at Poamoho, Oahu, in 1944*

Rate of application in pounds per acre	Nematode infestation						Yield in pounds per plot
	Percentage of plants in each class					Mean class value	
	None	Very slight	Slight	Moderate	Severe		
	0	1	2	3	4		
Check	19.3	35.4	16.0	13.8	15.5	1.53	2.96
200 .	59.3	39.1	1.6	0.0	0.0	0.41	7.28
300 .	68.9	24.4	2.8	2.8	1.2	0.36	7.32
400 .	71.9	27.4	0.4	0.4	0.0	0.29	7.54
500 ..	79.6	20.4	0.0	0.0	0.0	0.19	8.22
Difference required for significance at 5 per cent level ...						0.58	2.54

needed on rate of application. The 1944 tests included treatments with 200, 300, 400, and 500 lb. D–D per acre. The fumigant was applied in a continuous band in open furrows and immediately covered with soil to a depth of 4 inches. The treatments were randomized and replicated 5 times. Seven days were allowed for fumigation and escape of the fumigant. Lettuce was then directly seeded in the furrows just above the point of application of the fumigant. The plants were harvested at the stage of maximum head development. Yields were determined and the plants classified for nematode infestation. The results are in table 4.

Nematodes were not completely eradicated by any of the treatments. The population was greatly reduced, however, as was indicated by the infestation ratings and by the increase in yield following treatment. The yield of lettuce was more than doubled with all rates of application. There were no significant differences between treatments.

Depth of application. The influence of depth of D–D application was determined at Poamoho, Oahu, using lettuce as the test plant. D–D was

TABLE 5.- *Influence of the depth of application of D-D on root-knot development, and on the yield of lettuce at Poamoho, Oahu, in 1944*

Depth of application	Nematode infestation				Mean class value	Yield in pounds per plot
	Percentage of plants in each class					
	None 0	Slight 1	Moderate 2	Severe 3		
Check	6.1	25.5	47.2	21.2	1.85	7.4
4 inch	59.3	39.1	1.6	0.0	0.43	14.4
6 inch	66.7	22.1	0.8	0.4	0.35	13.0
8 inch	64.0	34.7	1.0	0.0	0.37	15.4
Difference required for significance at 5 per cent level					0.31	5.2

applied with a Vermorel injector at the rate of 300 lb. per acre at 4-, 6-, and 8-inch depths. Lettuce was directly seeded one week following fumigation. The lettuce was harvested when the heads were at maximum development. Yields and severity of nematode infestation were determined. The results are in table 5.

As in earlier experiments the nematode infestation was greatly reduced through treatment with D-D. Differences in lettuce yields or nematode infestation were not found among the three depths of application.

Methods of application. Three methods of D-D application were compared at Poamoho, Oahu, in 1944. D-D was (1) injected with a Vermorel injector, (2) applied in a continuous band, and (3) applied in the irrigation water as an emulsion. The rate of application was 300 lb. per acre. Lettuce was grown as an indicator crop. The results are in table 6.

The injection and continuous band methods of application were equally effective in controlling nematodes as is shown by the reduction in infestation and by the increase in yield. Applying D-D as an emulsion in the irrigation water gave no better results than the check.

1945

Results with D-D have shown that its effectiveness as a soil fumigant is influenced by soil conditions at the time of application. The purpose of the

TABLE 6.—*Influence of the method of application of D-D on root-knot development and on the yield of lettuce at Poamoho in 1944*

Method of application	Nematode infestation					Mean class value	Yield in pounds per plot
	Percentage of plants in each class						
	None 0	Very slight 1	Slight 2	Moderate 3	Severe 4		
Check	7.7	44.4	21.8	16.2	1.0	1.73	4.1
Injection	76.2	22.9	0.4	0.4	0.0	0.25	10.8
Continuous band	41.3	41.7	10.0	4.3	2.6	0.83	9.8
In water	21.0	51.0	13.4	9.5	5.1	1.18	4.6
Difference required for significance at 5 per cent level						0.76	3.1

1945 tests was to determine the effectiveness of D–D in a porous soil with a high temperature. The test area selected was at Koko Head, Oahu, at an elevation of approximately 15 feet above sea level. The soil was porous, showing very little evidence of lateritic weathering, and had an extremely heavy nematode population. A tomato crop had just been removed, and undecomposed root galls were still present. The area was thoroughly plowed and disced before treatment. D–D was applied at the rates of 100, 200, and 400 lb. per acre. The treatments were replicated 4 times and were arranged in a Latin square. The D–D was applied in the plow furrow by means of a burette and was covered with the plow immediately following application. The moisture content as determined[3] from representative samples collected from approximately the 3-inch depth averaged 16.9 per cent at the time of D–D application. The total porosity averaged 49.6 per cent and the organic content 3.48 per cent at the time of application. The soil pH ranged from 5.8 to 6.3. The soil temperature was 86° F. at the 3-inch depth and 84° F. at the 6-inch depth.

Manoa lettuce was used as the test crop. Plantings were made 1 day, 3 days, and 7 days following fumigation to determine the persistence of the toxic effects. The plantings were randomized within the replications. The lettuce was directly seeded and was thinned to 9 inches between plants following emergence. Irrigation was by the furrow system but water was not permitted to flow from one treatment to another. The lettuce was harvested at the stage of maximum head development. Thirty plants were harvested from each plot together with the root systems. The roots were removed, classified for nematode infestation, and weighed. The lettuce heads from each plot were also weighed. The results are in tables 7 and 8.

TABLE 7.—*Effect of the lapse of time between application of D–D and planting on the yield, root weight, and nematode infestation of lettuce at Koko Head, Oahu, during the summer of 1945*

	Pounds of D–D per acre	Time between treatment and planting		
		One day	Three days	Seven days
Yield per plot, in pounds	Check	5.0	7.1	6.6
	100	5.2	7.6	5.2
	200	6.2	10.0	7.4
	400	11.3	11.9	10.9
Weight of roots, in grams	Check	133.0	137.8	129.5
	100	157.0	159.3	124.8
	200	150.8	180.8	137.8
	400	187.5	191.0	178.6
Nematode infestation index	Check	94.2	97.2	97.5
	100	90.7	98.3	99.7
	200	76.1	82.6	89.8
	400	57.5	66.9	67.5

[3] The soil moisture, porosity, organic content, and pH determinations were made by the Chemistry and Soils department of the University of Hawaii Agricultural Experiment Station.

TABLE 8.—*Influence of soil fumigation with D–D on the yield, weight of roots, and nematode infestation of lettuce grown at Koko Head, Oahu, in the summer of 1945*

Rate of application in pounds per acre	Yield in pounds per plot	Weight of roots in grams	Nematode index
Check	18.7	400	96.1
100	18.0	441	96.2
200	23.6	469	83.1
400	34.0	557	64.0
Difference required for significance at the 5 per cent level	1.0	88.0	4.0

Inhibitory effects were not observed even when lettuce was seeded the day following treatment (Table 7). The yield, root weight, and nematode infestation were similar for the 3 dates of seeding. The plants from the 3 dates of planting were combined from each replication when harvested and the resulting data analyzed statistically. The 100-lb. application did not control nematodes and resulted in no increase in yield. The 200-lb. application resulted in both a reduction in nematode infestation and an increase in yield. The yield following the 400-lb. application was approximately twice that of the check. Although the 200- and 400-lb. applications reduced the nematode infestation, the nematodes were not eradicated as is indicated by the high nematode indexes following these treatments.

SOIL COVERS AND THEIR EFFECT ON FUMIGATION

When applying a soil fumigant, the recommendation is frequently made that a soil cover be used to prevent the rapid escape of the fumigant. Several types of covers were tried in 1945 under uniform conditions in artificially infested beds. The beds were 10 feet long, 4 feet wide, and 18 inches deep. The walls were constructed of concrete tile, and the soil used was a mixture of 2 parts garden soil and 1 part coral sand. The pH of the soil was between 7.6 and 7.9, and the soil moisture at the time of fumigation was 14.3 per cent in the upper 2 inches and 16.1 per cent at the 6-inch depth. The beds were infested by thoroughly mixing 4 lb. of chopped, heavily galled tomato roots into the soil of each bed one week in advance of planting.

The D–D was applied at 1-foot intervals at a depth of 6 inches by means of a burette. Three and one-half cubic centimeters were applied per square foot which very closely approximated a 400-lb.-per-acre rate. Each bed was divided into 2 parts by means of a heavy board, thereby permitting 2 treatments to the bed. After the D–D had been applied the beds were given the following treatments: (1) Bed covered with glue coated paper. (2) Bed covered with 3 layers of newspaper and lightly wetted down twice a day. (3) Bed soaked with water twice a day. (4) Bed wet down to a depth of 2 to 3 inches. (5) No cover or water seal. (6) Check. No cover or D–D treatment.

The paper covers were removed and watering discontinued after the fifth day. Bounty tomato seed was planted in rows spaced 7 inches apart one

week following treatment. When the tomato seedlings were 5 weeks old they were removed and the roots examined for nematode gall formation. An examination of a minimum of 200 plants per treatment showed no nematode gall formation on the roots of plants from covered or watered beds. One small gall was observed on a single plant from the treatment receiving no cover or water seal. Plants removed from the nontreated check showed severe root galling and were less than half the size of the plants from fumigated beds.

EFFECT OF D–D ON THE INCIDENCE OF BACTERIAL WILT

An observation during the 1943 tests indicated that application of D–D might increase the incidence of bacterial wilt of tomatoes. To determine the validity of this observation the influence of D–D and chloropicrin on the incidence of the disease was measured under controlled conditions. A mixture of $2\frac{1}{2}$ parts of garden soil and 1 part coral sand was placed in a tile-enclosed bed and heavily inoculated with a water suspension of *Bacterium solanacearum* grown in nutrient broth. Tomato plants grown in the soil were killed by bacterial wilt. Samples of soil were removed, placed in sealed drums, and fumigated with 7 different amounts of D–D and chloropicrin as listed in table 9. After allowing one week for fumigation, eight 1-gallon cans of soil were removed from each drum. Six tomato seedlings were transplanted to each can. The occurrence of bacterial wilt is recorded in table 9.

TABLE 9.—*Influence of D–D and chloropicrin on the incidence of bacterial wilt, Bacterium solanacearum, on tomatoes grown in the greenhouse in artificially inoculated soil at Honolulu, Oahu, in 1945*

Application in pounds per acre	Percentage incidence at 4 dates following transplanting[a]			
	19 days	27 days	38 days	55 days
Check	62.5	89.6	95.8	100.0
100 lb. D–D	31.3	66.7	81.3	91.7
200 lb. D–D	22.9	52.1	77.1	85.4
400 lb. D–D	6.3	18.8	31.3	91.7
600 lb. D–D	0.0	4.2	10.4	52.1
1000 lb. D–D	0.0	2.1	4.2	14.6
400 lb. chlor.	2.1	2.1	6.3	27.1
400 lb. chlor., 400 lb. D–D	0.0	0.0	2.1	20.8

[a] Determined from 48 plants grown in gallon cans (6 plants to the can).

All treatments reduced the occurrence of bacterial wilt as compared to the check. None of the treatments eradicated the organism, however. Chloropicrin at the 400-lb.-per-acre rate was more effective than D–D at the same rate. D–D at the 1000-lb. rate was the most effective of the treatments. With the 1000-lb. rate, an inhibitory effect on growth was noted for about 30 days following transplanting. The plants receiving a combination of D–D and chloropicrin were the most vigorous throughout the test.

DISCUSSION

In Hawaii the yield of vegetables has been increased with applications of D–D as low as 200 lb. per acre. The nematocidal properties of D–D are

comparable to those of chloropicrin, which is more costly and difficult to apply. Effectiveness of the fumigation is dependent on the soil type and condition, on environmental factors, and on the thoroughness of the application.

The results show that a definite recommendation regarding the rate of application cannot be made. A lower rate was required with the heavier soils at Poamoho than with the lighter, more porous soils at Koko Head. This difference in efficiency may also be partially attributed to rapid volatilization of D-D at the high soil temperatures which are prevalent at the lower elevations particularly during the summer months. The manufacturers have warned that effectiveness may be reduced at temperatures above 80° F.

Results from tests in tile-enclosed beds indicate that effective nematode control may be obtained without the use of a soil cover or seal following fumigation. Soil covers were not tried on soils with extremely high temperatures such as are found during the summer at the lower elevations. It might be expected that a good seal would retard the rapid escape of the fumigant under these conditions and thereby increase the efficiency of the fumigation.

The favorable yield response obtained with D-D is undoubtedly due to a combination of factors. Although the root-knot nematode infestation was reduced each time an improvement in yield was noted, the reduction was not always in proportion to yield increase. No study was made of biological responses other than nematode control and the preliminary study of Fusarium wilt and bacterial wilt control.

No data were secured on the persistence of treatment effects. In none of the field tests were all of the nematodes completely eradicated, however. Under the favorable Hawaiian environment the nematode population may be expected to build up rapidly when susceptible crops are grown. Other beneficial results may be more persistent in their effect.

SUMMARY

Yields of vegetables were increased and the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, thoroughly controlled with D-D applications as low as 200 lb. per acre when conditions for fumigation were favorable. Under less favorable soil and environmental conditions the nematodes were only partially controlled with applications of 400 lb. per acre. The required rate of application was shown to be higher in a warm (84°–86° F.), porous soil than in a heavy, compact soil with a lower temperature. The continuous band and spot injection methods of application gave similar results. D-D applied as an emulsion in the irrigation water was ineffective. No differences were noted in the effectiveness of D-D with the shallow-rooted lettuce plant when the depth of application ranged between 4 and 8 inches. The nematocidal properties of D-D were found to be similar to those of chloropicrin.

The phytocidal effect of D-D was unimportant as was demonstrated by the absence of injury even though all the field plantings were made within a week following treatment. Beneficial effects from soil covers or seals were not demonstrated from tests made in artificially inoculated test beds.

The incidence of bacterial wilt, *Bacterium solanacearum*, was reduced in the greenhouse by soil fumigation with both D–D and chloropicrin. D–D was less effective than chloropicrin.

The root-knot nematode was not completely eradicated in any of the field treatments.

DEPARTMENT OF VEGETABLE CROPS,

UNIVERSITY OF HAWAII

AGRICULTURAL EXPERIMENT STATION.

LITERATURE CITED

1. CARTER, WALTER. A promising new soil amendment and disinfectant. Science **97**: 383–384. 1943.
2. ——— ———. The use of D–D mixture against *Anomala* and *Adoretus* infesting nursery stock. Jour. Econ. Ent. **37**: 117. 1944.
3. ——— ———. Soil treatments with special reference to fumigation with D–D mixture. Jour. Econ. Ent. **38**: 35–44. 1945.
4. CHRISTIE, JESSE R. Some preliminary tests to determine the efficacy of certain substances when used as soil fumigants to control the root-knot nematode, *Heterodera marioni* (Cornu) Goodey. Proc. Helminth. Soc. Washington **12**: 14–19. 1945.
5. PARRIS, G. K. The nematocidal and fungicidal value of D–D mixture and other soil fumigants. Phytopath. **35**: 771–780. 1945.
6. TAM, R. K. The comparative effects of a 50–50 mixture of 1 : 3 dichloropropene and 1 : 2 dichloropropane (D–D mixture) and of chloropicrin on nitrification in soil and on the growth of the pineapple plant. Soil Science **59**: 191–205. 1945.

RESISTANCE TO CROWN RUST IN FESTUCA ELATIOR AND F. ELATIOR VAR. ARUNDINACEA[1]

K. W. KREITLOW AND W. M. MYERS[2]

INTRODUCTION

Crown rust, *Puccinia coronata* Cda., is one of the most destructive diseases of *Festuca elatior* L. in the northeastern United States. Although meadow fescue is a minor component of many hay and pasture mixtures, it can be grown almost anywhere in this region (5), consequently, it is found abundantly in meadows and along roadsides. General distribution of the host, therefore, offers ample opportunity for widespread attacks of the disease. Fortunately, the variety of crown rust that attacks *F. elatior* is restricted to several species of grasses and is seldom likely to attack oats (1, 3).[3] According to Brown (1) *Puccinia coronata* may be sub-divided into seven varieties depending upon grasses attacked. She concluded, as had several earlier workers, that var. *lolii* and var. *festucae* are difficult to differentiate and are probably physiologic races of the same variety of rust. In the present experiments no effort was made to differentiate the varietal or physiologic forms of *P. coronata*.

In recent years a coarse-leaved, large growing fescue known as "tall" fescue [*F. elatior* var. *arundinacea* (Schreb.) Wimm.] has been utilized as a forage grass. Tall fescue, although not tested so extensively as meadow fescue, has been observed by Piper (5) and Hardison (2) to be highly resistant to crown rust. As a result of preliminary observations and greenhouse tests a program of selection and breeding was initiated to secure resistance to crown rust in plants of *F. elatior*.

MATERIALS AND METHODS

Single tillers were usually established from plants moved from the field to the greenhouse for further testing. Most of the clones were tested in triplicate to reduce variation in infection to a minimum. Plants were also established from seeds of different collections by sowing them in flats of steamed soil and later transplanting individual seedlings to soil in 3-inch pots.

Urediospores were obtained in several ways. Leaves with mature uredia were collected in the field and stored in a refrigerator. Urediospores were also collected from leaves of heavily rusted plants by gently shaking the plants over a large evaporating dish. The spores were transferred to vials

by means of a camel's hair brush and stored in a refrigerator until used. Heavily rusted plants provided another source of inoculum since they were easily transplanted from the field to pots in the greenhouse. Abundant uredial infection was maintained on leaves of susceptible plants by periodically rubbing diseased and healthy leaves and then incubating the plants in a moist chamber.

For inoculation purposes, plants were atomized with a composited suspension of urediospores in tap water, and the inoculated plants were then incubated 48 hr. in a moist chamber. After removal from the moist chamber the plants were placed on a bench in the greenhouse for development of uredia. Severity of rust and infection type were read 10 to 15 days following inoculation. Murphy's classification of infection types for crown rust of oats (3) was adopted with slight modifications. All very susceptible plants were placed in class 3. This included all plants having abundant, moderately large to large uredia with or without necrosis or chlorosis immediately surrounding the uredia. Most susceptible plants observed developed chlorotic areas immediately surrounding the uredia. Plants that reacted doubtfully to inoculation were clipped and reinoculated after new noninfected leaves developed.

RESULTS

During the summers of 1942 and 1943, spaced plants of *Festuca elatior* and *F. elatior* var. *arundinacea* were observed for resistence to crown rust. Of several thousand plants of *F. elatior* examined in the nursery only a few showed evidence of resistance. The resistant clones were transplanted to the greenhouse and tested further during the winter of 1943 and 1944. Artificial inoculation confirmed field observations that clones of *F. elatior* in general are susceptible to crown rust while clones of *F. elatior* var. *arundinacea* are usually resistant. However, several plants of *F. elatior* observed to be free of rust in the field proved immune when tested in the greenhouse. Seeds which produced the rust-immune plants (247–1 and 247–2) had been collected by Doctor F. H. Steinmetz, Department of Botany, University of Maine, in a meadow near Orono, Maine. In figure 1, healthy leaves from a rust-immune plant of *F. elatior* are compared with rusted leaves from a susceptible plant.

Additional seeds were collected by Doctor Steinmetz from plants growing in this meadow and adjacent fields during the summer of 1944. Plants obtained from these seeds as well as plants secured from several other seed collections were tested for susceptibility to crown rust during the following winter.

Cytological studies are being reported in detail in another paper, by Myers and Hill (4). Hence only the data on chromosome numbers will be presented here (Table 1).

Data presented in table 1 came from testing approximately 20 plants of each strain of fescue derived from single seeds of a collection. In most
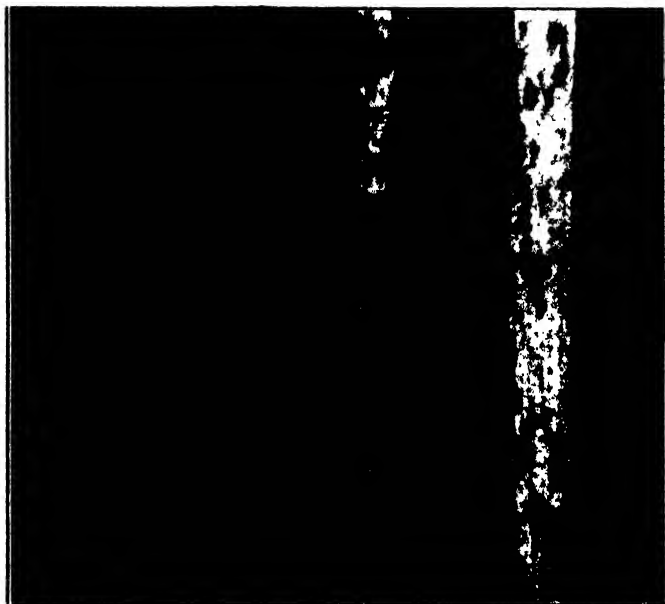
FIG. 1.   Leaves from a rust-immune plant of *Festuca elatior* on the left and a rust-susceptible plant on the right.

cases, the 14-chromosome *F. elatior* was susceptible to crown rust while the 42-chromosome *F. elatior* var. *arundinacea* was resistant.   Both the Svälof and Otofte strains yielded plants of variable rust reaction.   Some plants appeared resistant to rust while others were extremely susceptible.   However, none of the resistant plants had the immunity to crown rust characteristic of the Maine rust-immune plants.   Variability within the Svälof and Otofte strains suggests that crown rust-resistant individuals might be selected from this material.

When plants obtained from seeds of fescue harvested in the vicinity of the original Maine rust-immune plants were tested, no further 14-chromosome, immune plants were discovered.   A summary of the rust reactions of

TABLE 1.—*Crown rust reaction and chromosome number of some collections of Festuca elatior and F. elatior var. arundinacea*

Collection	Reaction to crown rust	2n chromosome number
Festuca elatior		
Maine 247–1 and 247–2	Immune	14
Svälof early strain	Resist. to susc.	14
Otofte strain	Resist. to susc.	14
Minn. 1449	Susceptible	14
S–53 (Wales)	Susceptible	14
F. elatior var. *arundinacea*		
N.Y. 2659 (England)	Immune to highly resistant	42
Alta (Ore. F.C. 29,366)	Immune to highly resistant	42
Suiter strain (Ky. K–31)	Immune to highly resistant	42

these plants is in table 2. One collection, (44-6), consisted of 42-chromosome plants which were preponderantly immune from crown rust; the other collections were 14-chromosome plants which were mostly susceptible.

TABLE 2.—*Crown rust reaction and chromosome number of three fescue collections from Maine*

Collection	Number of plants in each rust reaction class[a]					Total	$2n$ chromosome number
	I	0	1	2	3		
Maine 44-6 (27 heads collected from meadow near original pasture)	1130	31	135	5	12	1313	42
Maine 44-7 (10 heads collected from old lawn adjacent to original pasture)	0	1	0	5	282	288	14
Maine 44-8 (8 heads collected from original pasture)	0	0	1	25	214	240	14

[a] I—Immune—no macroscopic evidence of infection.
0—Nearly immune—no uredia formed; necrotic areas or chlorotic flecks present.
1—Highly resistant—no uredia or uredia few, small, always in necrotic areas; necrotic areas often produced without development of uredia.
2—Moderately susceptible—uredia fairly abundant, small to midsized, always in necrotic or chlorotic areas.
3—Susceptible—uredia abundant, midsized to large with or without necrosis or chlorosis immediately surrounding the uredia.

DISCUSSION

It has been shown experimentally that 14-chromosome *Festuca elatior* is usually susceptible to crown rust, *Puccinia coronata*, while 42-chromosome *F. elatior* var. *arundinacea* is usually resistant. That some rust-resistant germ plasm exists in *F. elatior* is demonstrated by the variability in rust reaction encountered in the varieties Svälof and Otofte. Since only one collection of rust-immune *F. elatior* was encountered among all the material tested the natural occurrence of rust-immune *F. elatior* appears to be rare. Additional collections secured from the same and neighboring fields proved to be either *F. elatior* in which most of the plants were susceptible to crown rust or *F. elatior* var. *arundinacea* in which most plants were resistant. The 14-chromosome condition of the rust-immune material should aid in transferring rust resistance to strains of *F. elatior* that are agronomically desirable but susceptible to *Puccinia coronata*.

SUMMARY

Most collections of meadow fescue, *Festuca elatior*, are susceptible to crown rust, *Puccinia coronata*, while those of tall fescue, *F. elatior* var. *arundinacea*, are usually resistant.

One collection of meadow fescue obtained from Maine was immune from crown rust.

Additional material from the same and neighboring fields proved to be either meadow fescue in which the majority of plants were susceptible to

crown rust or tall fescue which varied in rust reaction from susceptible to immune.

U. S. REGIONAL PASTURE RESEARCH LABORATORY,
STATE COLLEGE, PENNSYLVANIA.

LITERATURE CITED

1. BROWN, MABLE R.   A study of crown rust, *Puccinia coronata* Corda, in Great Britain. I.   Physiologic specialization in the urediospore stage.   Ann. Appl. Biol. 24: 504–527.   1937.
2. HARDISON, JOHN R.   Observations on grass diseases in Kentucky, September, 1942, to September, 1944, and a preliminary check list.   U. S. Dept. Agr., Pl. Dis. Reptr. 29: 76–85.   1945.
3. MURPHY, H. C.   Physiologic specialization in *Puccinia coronata avenae*.   U. S. Dept. Agr. Tech. Bull. 433.   1935.
4. MYERS, W. M., and HELEN D. HILL.   Distribution and nature of polyploidy in *Festuca elatior* L.   (In manuscript.)
5. PIPER, C. V.   Forage plants and their culture.   Macmillan Co., New York.   1924.
6. SEMPLE, A. F., H. N. VINAL, C. R. ENLOW, and F. E. WOODWARD.   A pasture handbook.   U. S. Dept. Agr. Misc. Publ. 194.   1934.   Rev. 1942.

PHYTOPATHOLOGICAL NOTES

Pinto Leaf, a Transmissible Disease of Cherry.—During a survey of cherry orchards at The Dalles, Oregon, in June, 1943, two Napoleon (Royal Ann) trees were discovered with unusual chlorotic leaves. Since the mosaic symptoms on the diseased leaves resemble the blotchy pattern of certain western horses known as pinto ponies, the name "pinto leaf" has been selected as a descriptive common name. In subsequent surveys at The Dalles the disease also was found on the varieties Napoleon, Black Republican, and Stark Gold, as well as on mazzard seedlings, and later two infected mazzard seedling trees were discovered at Hood River, Oregon. Pinto leaf is of minor economic importance at the present time because of its limited occurrence.

Large to small chlorotic patches form on diseased leaves. The original pale green to yellow color of diseased tissue gradually changes to bright yellow or white. Any part of the leaf may be affected, but the chlorosis rarely forms a specific pattern (Fig. 1, A). Leaf symptoms are sometimes obscure or meager, especially on mazzard seedlings, in which the chlorosis often appears as a coarse stippling. Leaves of terminal shoots rarely become chlorotic, but when this occurs a few of the basal leaves may show signs of infection late in the season. Severely diseased trees appear slightly dwarfed, produce less new growth, and the foliage appears somewhat ruffled when viewed from a distance.

The fruit of Napoleon and Stark Gold on affected trees never attained proper maturity. When healthy Napoleon fruits were fully colored and sweet, diseased fruits remained yellowish-green and insipid in taste. The fruit from affected Napoleon trees had rarely been picked, according to the owner, because of its lack of color and inferior quality. Infected Stark Gold yielded smaller fruit of inferior flavor. The color differences have not been noticeable in any infected black cherry variety, but trees with symptoms as severe as in the Napoleon variety have not yet been observed.

Buds from diseased pinto-leaf trees were inserted into 13 symptomless sweet-cherry seedlings late in June, 1943. Definite symptoms of pinto leaf appeared in 3 of the seedling trees during the spring of 1944. In a second attempt at transmission during August, 1944, a series of buds taken from symptomless terminal shoots of diseased trees was inserted at Hood River into 20 healthy mazzard seedlings. A second group of 24 seedling trees was budded with infected heel spurs that had produced visibly infected leaves. Only one seedling of the first group became infected although several of the original buds formed new growth with clean foliage. Of the "spur bud" group, 5 seedlings developed typical symptoms, indicating that the virus probably is more concentrated in older growth or that it moves into terminal shoots rather tardily. Transmission to the seedling trees was apparent early in the spring of 1945.
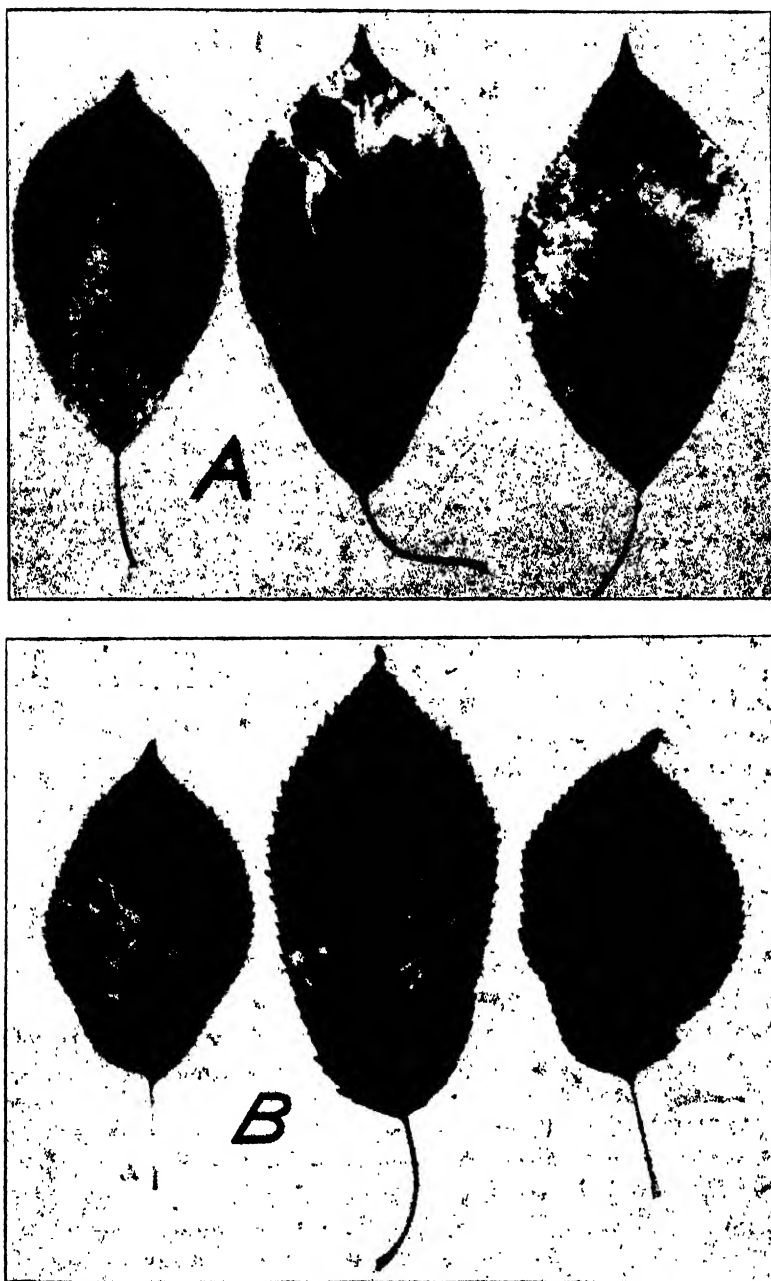
FIG. 1.   Pinto leaf virus symptoms compared with "white spot" on cherries.   A. Pinto leaf on Napoleon cherry leaves.   B. White spot, a nontransmissible malady, on Black Republican cherry leaves.

The pinto-leaf symptoms on cherry resemble apple mosaic,[1] grape mosaic,[2] peach calico,[3] and a "white spot" disease of cherry,[4,5] but are distinct from line pattern as reported on cherry.[6] The characteristic "oak leaf" pattern of white spot (Fig. 1, B) generally serves to distinguish it from pinto leaf. Buds from typical white spot found on a Black Republican tree at The Dalles failed to transmit or perpetuate the symptoms when worked onto mazzard seedlings. The results substantiate those of Thomas and Rawlins[5] and Cochran[7] that white spot is not an infectious disease.

Attempts were made to transmit pinto leaf to sour cherry, peach, plum, apricot, chokecherry, rose, apple, and pear. A few fugitive leaf symptoms were observed only on one peach seedling, but they resembled neither those of cherry pinto leaf nor of peach calico. Under dry land farming conditions existing at The Dalles, successful bud unions form with difficulty. and this may account, in part, for the relatively small number of successful transmissions in that district.

Pinto leaf symptoms are distinct from any known disease affecting sweet cherries. For those desiring a Latin binomial, the name *Marmor pinto-folium* is proposed.—J. R. KIENHOLZ, U. S. Fruit Disease Laboratory, Hood River, Oregon.

Virus Transmission by Cuscuta sandwichiana.[1]—Since Bennett[2] published the first paper on the subject in 1940, *Cuscuta subinclusa* Dur. and Hilg.,[2] *C. californica* Choisy,[2] and *C. campestris* Yuncker,[3,4] have been recorded as capable of transmitting plant viruses. The fourth species, *C. sandwichiana* Choisy, is reported here.

Cuscuta sandwichiana is an endemic species in Hawaii occurring occasionally along the coastal dunes or swamps. This dodder is not so aggressive a parasite as some of the other species and could not establish itself

[1] Bradford, F. C., and Lloyd Joley. Infectious variegation in the apple. Jour. Agr. Res. |U.S.| 46: 901–908. 1933.

[2] Hewitt, W. B. A graft-transmissible mosaic disease of grapevine. Phytopath. 35: 940–942. 1945.

[3] Blodgett, Earle C. Peach calico. Phytopath. 34: 650–657. 1944.

[4] Rhoades, A. S. Virus and virus-like diseases of sweet cherry in Utah, and notes on some conditions affecting various fruit crops. Plant Dis. Reptr. 29: 6–19. 1945.

[5] Thomas, H. Earl, and T. E. Rawlins. Some mosaic diseases of Prunus species. Hilgardia 12: 623–644. 1939.

[6] Willison, R. S. A line-pattern virosis of Shiro plum. Phytopath. 35: 991–1001. 1945.

[7] Dr. L. C. Cochran of this Division informed the writer he has made numerous attempts to transmit white spot in California without success.

[1] Published with the approval of the Director as Technical Paper No. 170 of the Pineapple Research Institute, University of Hawaii.

[2] Bennett, C. W. Acquisition and transmission of viruses by dodder (*Cuscuta subinclusa*). (Abstr.) Phytopath. 30: 2. 1940.

[3] Johnson, F. Transmission of viruses by the parasitic activities of dodder. (Abstr.) Phytopath. 31: 13. 1941.

[4] *C. repens* has also been reported by E. M. Hildebrand (The dodder vector of woody plant viruses. U. S. Dept. Agr., Pl. Dis. Reptr. 29: 196–197. 1945). The writer was informed by N. J. Giddings, who supplied the seeds to Hildebrand, that there had been some confusion in labeling and *C. repens* reported by Hildebrand should be known under the name *C. campestris*.

under greenhouse conditions. on several plants tested. These plants were
Dendrobium sp., Commelina diffusa Burm. f., *Emilia sonchifolia* (L.) DC.,
Nicotiana glutinosa L., and pineapple. It established with difficulty on
mature stems of tomato but never on young plants. It thrived, however, on
cucumber, *Boerhaavia diffusa* L., and *Asystasia gangetica* (L.) T. Anders.;
on the last plant a stock colony of the dodder was maintained.

The cucumber mosaic virus was very easily transmitted from cucumber
to cucumber when dodder growing on the infected plants was attached to
healthy plants. Twenty-one test plants were successfully infected. None
of 19 other test plants became infected when the terminal cuttings from
dodder grown on the infected plants were attached to them. It appears
that the Hawaiian dodder is not so efficient as other dodders in retaining the
virus. A mosaic, of which the identity has not been established, was com-
monly found on the island of Oahu on *Boerhaavia diffusa* which is an in-
digenous littoral herb. The virus was not transmitted into 29 test plants
through the dodder connection.

Repeated trials were all failures in transmitting the spotted wilt virus
from tomato to tomato. The dodder never established on young tomato
plants with fresh symptoms; however, it did on mature plants with old
symptoms. Forty-two mature test plants thus connected with such source
plants did not demonstrate successful virus transmission. Since the spotted-
wilt virus has been known to be difficult to acquire from the plants with
old symptoms through either sap or insect transmission, the present data
are not conclusive. The spotted-wilt virus, however, appears to be com-
paratively difficult to transmit through dodder vectors, as Bennett[5] reported
this to be the case with *Cuscuta subinclusa, C. californica,* and *C. campestris,*
particularly through the first two species.

Another indigenous parasitic vine, *Cassytha filiformis* L., which is much
coarser than *Cuscuta,* has been proved to be unsatisfactory for transmission
work on herbaceous plants.—K. SAKIMURA, Pineapple Research Institute,
Honolulu, Hawaii.

Sclerotium rolfsii Sacc. and its Perfect Stage on Climbing Fig.—Climb-
ing fig (*Ficus pumila* L.) is commonly used in Florida to cover stone or
brick walls because it grows rapidly, is evergreen and is seldom attacked
by insect pests or fungus diseases. During July, 1945, *Sclerotium rolfsii*
Sacc. was found on several areas of dead leaves, noted on the north wall of
the Plant Pathology greenhouse at Gainesville. These areas were roughly
semi-circular with a radius of 6 to 18 inches and with the flat side of the
semicircle at the ground. Mycelium was especially conspicuous on the
tender, green stems and the underside of the leaves. White to pale mycelium
covered the affected host organs, fastening adjacent leaves together in some
cases and spreading out fan-like over newly affected leaf areas. Small,

[5] Bennett, C. W. Studies of dodder transmission of plant viruses. Phytopath. **34:**
905–932. 1944.

nearly spherical sclerotia were formed on the dead, young stems and along
the edges of affected leaves.   Most of the dead leaves were reddish-brown,
although those most recently affected were brownish-olive.   Young stems
and leaves were killed outright.   Older stems, from which the leaves had
fallen, were not killed, and they produced new sprouts later in the season.
Cultures on potato-dextrose agar, using either sclerotia or pieces of the
advancing margin of the mycelium as inoculum, were typical of the fungus
on this medium and produced numerous tan sclerotia about 1 mm. in diame-
ter.
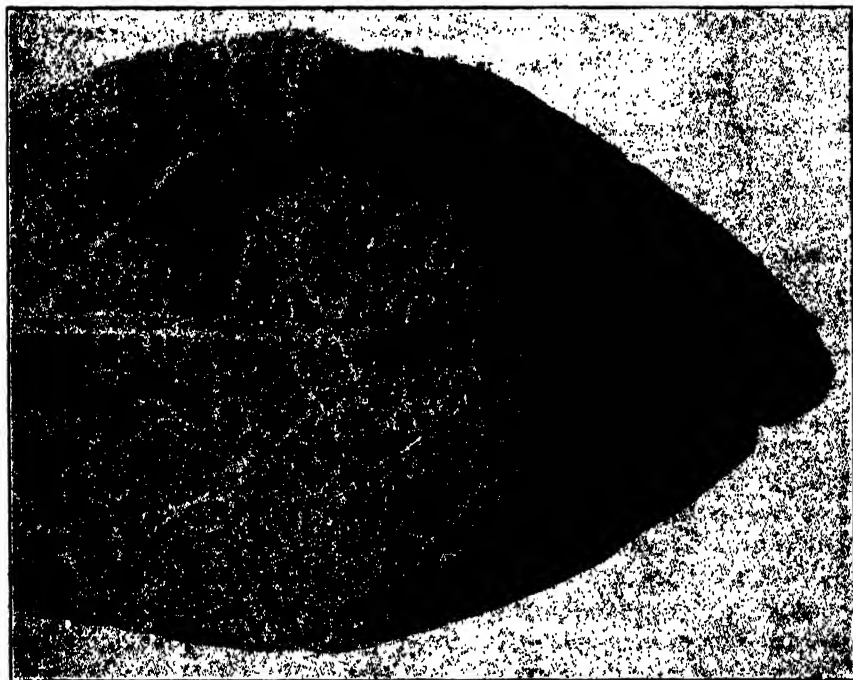
FIG. 1.   Hymenium of *Pellicularia rolfsii* on climbing fig leaf.   (Mag. × 5.)

While material was being collected for a permanent record of the fungus
on this new host, a basidial form was discovered on a few leaves.   This was
found in considerable quantity later when 2 or 3 sunny days followed pro-
longed (4- to 6-day) rainy periods.   It occurred only on the underside of
leaves (Fig. 1) always on the shaded side, always on leaves in the middle
or inner layers of vines, and usually several inches behind the actively
advancing margin of the fungus.   A few patches of this basidial stage
occurred in June and July of 1946 on leaves in the same general area.

The hymenium is very coarsely areolate at first, consisting of clusters
of basidia arranged in lines on a very tenuous subiculum.   As more basidia
are formed, the hymenium becomes more dense but never forms a continuous
or fleshy layer, and, when fully developed, it is not more than 30 to 40 µ in

thickness. The color of the fruiting hymenium is putty (M. & P. 11–B–2)[1] but in herbarium specimens a year old, it is more gray (M. & P. 42–A–2 or 41–A–2).[1] The basidia are obovoid, 7 to 9 μ long by 4 to 5 μ wide. Each basidium bears 2 or 4 parallel or divergent sterigmata, 2.5 to 4 or occasionally 6 μ long. The spores are elliptical to obovate, rounded above, rounded or pointed at the base, apiculate, 3.5 to 5 μ by 6 to 7 μ, hyaline and smooth. No cystidia or incrusted hyphae have been found.

Two species of *Corticium* have been reported as the basidial stage of *Sclerotium rolfsii* Sacc., namely *Corticium centrifugum* (Lev.) Bres. (reported by Goto[2] and Curzi[3]) and *C. rolfsii* (Sacc.) Curzi (reported by Curzi,[3] Milthorpe,[4] Barrett,[5] and Mundkur[6]). The spore measurements of the *Corticium* on fig fall within the limits given for *C. centrifugum* but such characteristics as color and thickness of the hymenium are quite different. The basidia, sterigmata, and spores agree very well with those described for *C. rolfsii* and since the other characters of that species were described from material on culture media and hence are hardly comparable to naturally produced fructifications, discrepancies here may be disregarded. For the present, the basidial stage on the fig leaves is regarded as *Corticium rolfsii* (Sacc.) Curzi, but inasmuch as Corticium species having an areolate hymenium, short-celled, stout hyphae, right-angled branching of the mycelium and stout basidia have been segregated in the genus *Pellicularia*, the combination *Pellicularia rolfsii* (Sacc.) nov. comb. is proposed as the correct name.

Fifteen single-basidiospore cultures, obtained by suspending fresh hymenia on fig leaves over dishes of potato-dextrose agar, have been compared and considerable variations were noted in color, marking, size, and frequency of the sclerotia and in characteristics of the mycelium growth. Some isolates resembled typical *Sclerotium rolfsii* Sacc., others resembled *S. delphinii* D. S. Welch, and some were intermediate. All isolates are parasitic on *Lupinus angustifolius* L.—ERDMAN WEST, Florida Agricultural Experiment Station, Gainesville, Florida.

Aster Yellows and Its Vector on Potatoes in Nebraska.—In recent years it has been stated[1, 2] that aster yellows is transferable to potatoes by the aster

[1] Maerz, A., and M. Rea Paul. A Dictionary of Color. 1st Ed., 207 + VII pp. McGraw-Hill. New York. 1930.

[2] Goto, K. On the perfect stage of *Sclerotium rolfsii* Sacc. produced on culture media. Preliminary report. Jour. Soc. Trop. Agric. 2: 165–175. 1930.

[3] Curzi, M. Studi su lo "*Sclerotium rolfsii.*" Boll. R. Staz. Pat. Veg., N. S. 11: 306–373. 1931.

[4] Milthorpe, F. L. Studies on *Corticium rolfsii* (Sacc.) Curzi (*Sclerotium rolfsii* Sacc.). Proc. Linn. Soc. N. S. W. 66: 65–75. 1941.

[5] Barrett, J. T. Observations on the basidial stage of *Sclerotium rolfsii.* Phytopath. 24: 1137–1138. 1934.

[6] Mundkur, B. B. Perfect stage of *Sclerotium rolfsii* in pure culture. Indian Jour. Agric. Sci. 4: 779–781. 1934.

[1] Leach, J. G. Further experiments on the cause of purple-top wilt of potatoes. Phytopath. 29: 14. 1939.

[2] Younkin, S. G. Purple-top wilt of potatoes caused by aster-yellows virus. Amer. Pot. Jour. 20: 177–183. 1943.

BOOK REVIEWS

BISBY, G. R. *An Introduction to the Taxonomy and Nomenclature of Fungi.* 117 pp. The Imperial Mycological Institute. Kew, Surrey, England. 1945. 5s or $1.25.

The sixteen chapters in this book are divided, following the introductory chapter, into: Part 1, Taxonomy, and Part 2, Nomenclature. Part 1 is mainly of interest to the beginning student in plant pathology or mycology. After addressing a chapter to the amateur, the writer discusses, in turn, how to choose a fungus group for study, the equipment needed, suggestions on collecting, examining, recording, measuring, and culturing fungi; naming and describing them; preserving material; and finally, suggestions on publishing and illustrating.

Part 2, which is considerably more technical than Part 1, has chapters on the categories of fungi, synonymy, types and the type method, diagnoses, rules of nomenclature, literature citations, and index. The value of this part of the book is greatly increased by the inclusion of the International Rules of Botanical Nomenclature, together with notes and examples of fungus names. As many plant pathologists have not had ready access to these rules, this feature alone is sufficient to recommend this inexpensive little book to the plant pathologist who works with fungi.—RODERICK SPRAGUE, Washington State College, Pullman, Washington.

DUBOS, RENÉ J. *The Bacterial Cell in Its Relation to Problems of Virulence, Immunity, and Chemotherapy.* 460 pp., 53 figs. Harvard University Press, Cambridge, Mass. 1945. $5.00.

This excellent book is Harvard University Monograph in Medicine and Public Health Number 6. Thus it deals almost exclusively with human pathogens. It covers the following general topics: (1) materials, problems, and methods; (2) cytology; (3) physicochemical and staining properties; (4) analysis of cellular structure by biochemical and biological methods; (5) variability; (6) nature of virulence; (7) immunization; (8) bacteriostatic and bactericidal agents; (9) trends and perspectives; and (Addendum, by C. F. Robinow) nuclear apparatus and cell structure of rod-shaped bacteria.

This book is really important for those interested in bacteria. However, it apparently contains only casual or no mention of numerous important items concerned with bacteria pathogenic to farm animals, with plant pathogens (4 references), with legume root-nodule bacteria (2 references), and with forms important in agriculture and industry.

The chapter on virulence is particularly noteworthy for the plant pathologist (but there is no mention of plant pathogens). Descriptions appear of (1) host-parasite relationships, (2) resistance of the parasite to the defense mechanisms, (3) factors affecting invasiveness, (4) toxic action, and (5) independent variation of the different components of virulence. The author emphasizes that virulence is not a single character but a combination of a number of different characters. These may vary independently. This neglected viewpoint deserves continued emphasis for those studying critical questions of virulence. It is complementary to questions of disease resistance.—A. J. RIKER, University of Wisconsin, Madison, Wisconsin.

GILMAN, JOSEPH C. *A Manual of Soil Fungi.* ix + 392 pp. 135 figs. The Collegiate Press, Inc., Ames, Iowa. 1945. $5.00.

In his preface Dr. Gilman states that this book is an expanded revision of the paper "A Summary of the Soil Fungi" published by Dr. E. V. Abbott and himself in 1927. The deserved popularity of that earlier contribution is a good index to the potential value of this present work. The compilation covers principally those soil fungi that have been isolated and grown on artificial media and excludes some special groups of fungi such as soil-borne plant pathogens not isolated directly from the soil. The often tedious task of identifying the fungi associated with soil is made easier by the many dichotomous keys, line drawings and adequate descriptions. Plant pathologists should welcome this book, for it brings into one volume descriptions of many of those fungi that occur not only in the soil but on seeds and other plant parts.—IAN W. TERVET, University of Nebraska, Lincoln, Neb.

AN ELECTRON MICROSCOPE STUDY OF MUTATION
IN TOBACCO–MOSAIC VIRUS

WILLIAM N. TAKAHASHI AND T. E. RAWLINS

Common tobacco-mosaic virus (Johnson's tobacco-mosaic virus 1) obtained from infective plant juice by differential centrifugation has a characteristic particle length of around 300 mµ (6, 8). Stanley (7) and Knight have made a number of contributions showing that at least certain virus mutations are accompanied by a change in the amino acid content of the virus. Melchers and co-workers (5) reported a strain of tobacco mosaic having a mean particle length of 137.5 mµ and another strain having a mean length of 187.5 mµ.

These reported differences between the particle length of common tobacco-mosaic virus and viruses which may be mutants of this virus bring up the possibility that certain mutations may be caused by fracture of the nucleo-protein virus particle. In an attempt to throw light on this question, we have used a virus that is presumably the result of a single mutation of common tobacco-mosaic virus. This virus is one of the yellow mosaics [McKinney (4) and Jensen (1)] which was obtained by isolating from the central region of one of the bright yellow spots that sometimes develop on leaves infected with common tobacco mosaic. The virus obtained from this spot was diluted and rubbed on *Nicotiana glutinosa* leaves to produce local lesions and to enhance its purity. The virus from one of the lesions was then inoculated into Turkish tobacco and produced chlorotic spots at the infection points on the inoculated leaves. These later became necrotic. The systemic symptoms consisted of a distinct yellowing along the veins, appearing first in the youngest leaves and remaining in the mature leaves. These symptoms have remained constant during several transfers of the virus through Turkish tobacco plants. The symptoms produced by this mutant are similar or identical with those illustrated by Stanley (7) as due to a strain of tobacco mosaic called J14D1.

The infected leaves were collected 20 days after inoculation and were frozen at –18° C. Purification was by the alternate high and low speed centrifugation method. The micrographs were taken with an R.C.A. Type B electron microscope. Both of these techniques have been described earlier (6).

Representative micrographs of common tobacco-mosaic virus and of the yellow mutant are in figure 1. The length distribution of the characteristic particles of the two viruses is shown in figure 2. It is evident that there were apparently no detectable changes in the size or appearance of the particle during mutation. Such changes as occurred during mutation were apparently not of a nature to be detected by means of the electron microscope.

Knight (2) has reported the rib-grass strain of tobacco-mosaic virus to have essentially the same particle size as tobacco-mosaic virus, and Knight
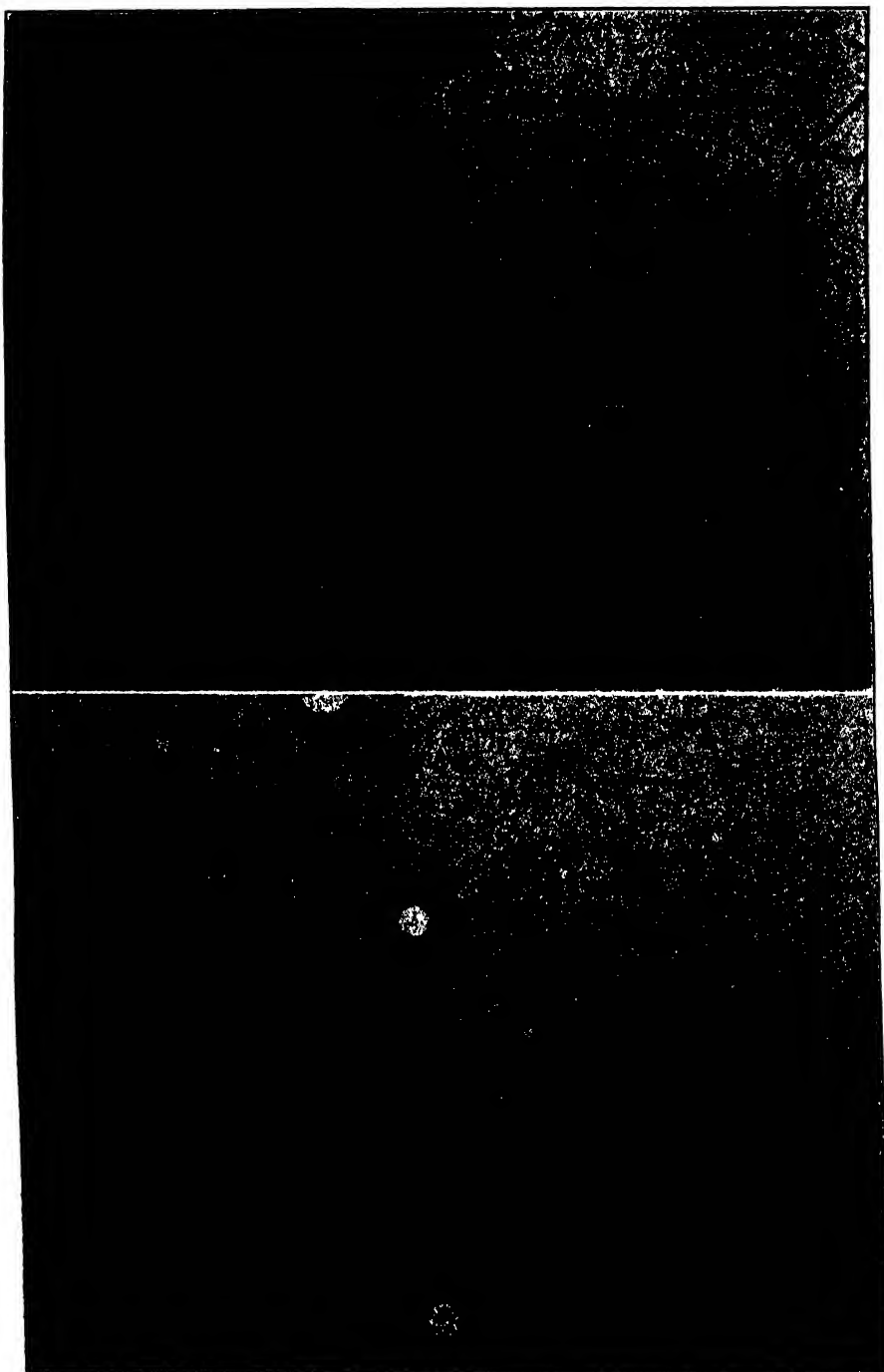
FIG. 1. Micrographs of two strains of tobacco-mosaic virus. A. Common tobacco-mosaic virus particles. B. Particles of a yellow mutant of tobacco-mosaic virus.

and Stanley (3) have reported cucumber virus 4 to have essentially the same size as tobacco-mosaic virus.   They have also presented other evidence showing that cucumber virus 4 may be related to tobacco-mosaic virus.   Unpub-
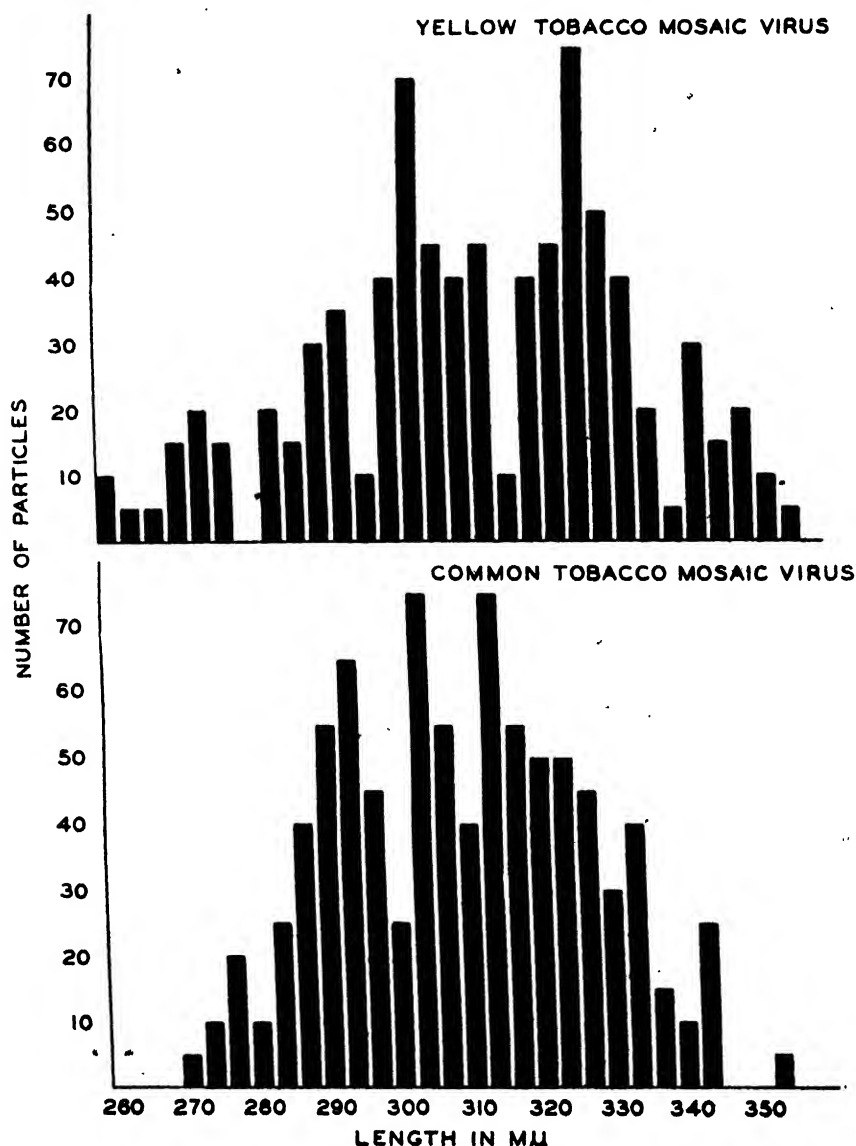

FIG. 2.   Length distribution curves of common tobacco-mosaic virus and a yellow-mutant of this virus.

lished results (9) indicate that the particles of 2 strains of potato X virus are usually between 500 and 600 mμ long and again are indistinguishable from each other in electron micrographs.   However, the symptoms produced by these 2 strains on tobacco show little resemblance.

Our results and those of Knight, and Knight and Stanley indicate that a detectable fracture of the virus particle does not ordinarily accompany mutations of rod-shaped viruses. At present there is unfortunately little evidence to indicate whether the changes in amino acid content, that have been found to accompany certain virus mutations (7), are a cause or a result of the mutations.

The results reported and reviewed above indicate that the size of a properly purified rod-shaped virus is a valuable character in determining the relationship of viruses.

In view of the fact that the 2 strains of tobacco mosaic reported by Melchers *et al.* (5) are the only reported strains where mutation appears to be accompanied by a shortening of the virus particles, the question arises as to whether these viruses were really strains of tobacco mosaic or whether their short particles may have been due to the methods of preparation.

SUMMARY

Common tobacco-mosaic virus and a yellow mutant isolated from it were studied with the electron microscope. The yellow mutant was indistinguishable in size and form from the parent strain when isolated from plant juice. It is concluded from the combined available evidence that mutations of rod-shaped viruses are ordinarily not accompanied by a modification of the virus particle sufficiently great to be detected by the electron microscope. Accordingly, if an unidentified virus has the particle size of a known rod-shaped virus there is considerable likelihood that the 2 viruses are related.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA.

LITERATURE CITED

1. JENSEN, J. H. Isolation of yellow-mosaic viruses from plants infected with tobacco mosaic. Phytopath. **23**: 964–974. 1933.
2. KNIGHT, C. A. The physical and chemical properties of a distinctive strain of tobacco mosaic virus. Jour. Biol. Chem. **145**: 11–18. 1942.
3. ————, and W. M. STANLEY. Preparation and properties of cucumber virus 4. Jour. Biol. Chem. **141**: 29–38. 1941.
4. MCKINNEY, H. H. Virus mixtures that may not be detected in young tobacco plants. Phytopath. **16**: 893. 1926.
5. MELCHERS, G., G. SCHRAMM, H. TRURNIT, and H. FRIEDRICH-FREKSA. Die biologische, chemische und elektronenmikroskopische Untersuchung eines Mosaikvirus aus Tomaten. Biol. Zentralbl. **60**: 524–556. 1940.
6. RAWLINS, T. E., CATHERINE ROBERTS, and NEDRA M. UTECH. An electron microscope study of tobacco mosaic virus at different stages of infection. Amer. Jour. Bot. **33**: 356–363. 1946.
7. STANLEY, W. M. Chemical structure and the mutation of viruses. Virus diseases. Cornell Univ. Press. 1943.
8. ————, and T. F. ANDERSON. A study of purified viruses with the electron microscope. Jour. Biol. Chem. **139**: 325–338. 1941.
9. TAKAHASHI, W. N., and T. E. RAWLINS. An electron microscope study of two strains of potato X virus. (In press.)

MILD RUSTY MOTTLE OF SWEET CHERRY (PRUNUS AVIUM)[1]

S. M. Zeller and J. A. Milbrath

INTRODUCTION

Mild Rusty Mottle of sweet cherry trees has been observed in Oregon for the last six or seven years. It was at first believed to be the same as the severe type of Rusty Mottle described by Reeves (5) from Washington, but comparative studies have proved it to be distinct in several respects. Mild Rusty Mottle occurs in some sections of the states of Oregon, Washington, and Idaho. In Oregon it has been observed throughout the Willamette Valley, and in Curry, Hood River, and Wasco counties. Where surveys have been made one orchard at least 35 years old had 20 per cent Mild Rusty Mottle while another orchard about 25 years old had 32 per cent of the trees infected (9). In some orchards, it has been estimated that more than 50 per cent of the trees were infected. On the other hand, some of the oldest orchards have little or none of the disease, and some young orchards may be relatively free of Mild Rusty Mottle, while still others originating from different nursery stock have only scattered trees infected from the start (3).

Although trees affected with Mild Rusty Mottle continue to produce fruit for a number of years, the loss from this disease in northwestern sweet cherry orchards is undoubtedly of considerable economic importance.

HOST RANGE AND SUSCEPTIBLE VARIETIES

Mild Rusty Mottle seems to infect one variety of sweet cherry about as readily as another. It has been found in the orchards affecting trees of the Bing, Black Republican, Black Tartarian, Lambert, and Napoleon varieties. It was also isolated from Montmorency sour cherry. A number of other stone fruit species and varieties have been found by inoculation to be susceptible. These will be discussed under transmission studies.

SYMPTOMS

In sweet cherries. From a distance one may distinguish trees affected with Mild Rusty Mottle by their general yellowish greenness as compared to the dark lush greenness of healthy trees. The whole tree takes on a rusty or bronzed appearance by late June or early July. Older infected trees often show considerable die-back and unthriftiness, indicating a probable increase with age in susceptibility to unfavorable climatic factors, such as drouth and low temperatures. Affected trees, however, seem to decline more rapidly than healthy trees, as indicated by poorer terminal growth, set of fruiting spurs, etc., although their decline is much slower than that brought about by Severe Rusty Mottle. The disease may be found in a single branch, but by the next season it may have spread to the whole tree.

There are no particular leaf symptoms very early in the spring. Usually in May or early June the mottling first appears in leaves which have attained a certain maturity some distance back from the growing tips. There are no symptoms in the terminal growth throughout the season.

The mottling starts as yellowish or merely light green areas in the leaves. These areas take on various forms. If circular, the centers are lighter yellow than the margins (Fig. 1, A), the latter gradually becoming bronzed or reddish (Fig. 1, B). This bronzing or rustiness shows first on the upper leaf surface, then gradually shows reddish below. It borders the areas whatever their shape or size. The bronzing or rustiness at times, however, is in the form of tiny stipples. This stippling may be only on, or very close to, the

FIG. 1. Three leaves illustrating stages in the development of leaf symptoms of Mild Rusty Mottle in Bing cherry; collected June 12, 1942, The Dalles, Oregon. A. Circular chlorotic areas constitute the first leaf symptoms. B. Rusty or bronzed margins form around the chlorotic areas. Such symptoms persist until late fall. C. A small percentage of the oldest leaves may take on a bright yellow rusty-mottled appearance and drop during June.

veins before it shows in the mesophyll. In such cases it usually starts at the base of the leaf blade. If the rustiness follows a line pattern or rings, there is usually a feathered out appearance, at least on one side. At times the leaves that come out early or are shaded toward the center of the tree may become chlorotic with a severe bright yellow or whitish mottle (Fig. 1, C). Such leaves are shed rapidly, but such shedding does not result in a general defoliated appearance of the tree. In unusual cases certain infected trees may have a rather heavy loss of relatively greenish mottled leaves even before fruit-harvest time, but the next year the same trees may have no particular leaf cast during the summer. Leaf symptoms of Mild Rusty Mottle differ little, if any, in different varieties of sweet cherries.

Fruits of trees infected with Mild Rusty Mottle are somewhat retarded in ripening. In the Napoleon variety the fruits are inclined to be clear yellow and usually without the red cheeks of healthy fruit.

In sour cherries. Montmorency sour cherry trees from which the Mild-Rusty-Mottle virus was obtained, had considerable die-back and a peculiar leaf symptom, neither of which has been definitely proved to be expressions of this particular disease. The leaves had a mild mottle and they were much smaller than on healthy appearing trees. The under surfaces of the leaves were mottled with a rusty coloration which seems to be brought about by necrosis of the lower epidermis in certain areas, and which may be limited to one side of the leaf, to the central area, or along the veins. A close examination of the rustiness shows it to start with a reddish necrosis along the veinlets. This spreads along these veins until the whole spot appears reddish to the naked eye. Finally the whole under surface of the leaf has a reddish mottle. By this time the upper surface appears mottled and necrosis begins in the older chlorotic spots.

TRANSMISSION STUDIES

Mild Rusty Mottle is perhaps as easily transmitted as any woody plant virosis. In every case where sweet cherry has been inoculated with sweet-cherry inoculum, transmission has been successful with or without apparent organic union of the two tissues. In all of our transmission experiments reported below, inoculation has been by summer budding. Sometimes, however, the inoculum has consisted of a mere shield of infected bark and wood slipped under the bark of the tree to be inoculated. Each graft is bound with a nurseryman's rubber band used in budding. In each experiment two to five trees of a variety were inoculated. The trees have ranged in age from one to five years from the nursery. There has been no indication of difference of reaction of young and older cherry trees to the Mild-Rusty-Mottle virus. All trees became uniformly infected the season following inoculation. Mild-Rusty-Mottle inoculum used in these studies came from the following localities: Benton, Hood River, Lane, Multnomah, Polk, Wasco, and Washington counties in Oregon, and Benton and Yakima counties in Washington.

SYMPTOMS IN INOCULATED TREES

More than 200 trees of several varieties of stone fruits have been inoculated with Mild Rusty Mottle and the results of these inoculations are reported rather concisely in table 1. Attention should be called to some of the peculiarities of the disease, as learned from the transmission studies.

In sweet cherries. The symptoms of Mild Rusty Mottle as obtained by graft inoculations are about equally conspicuous in the seven varieties of sweet cherries thus infected, unless it should be noted that sometimes in the leaves of Black Republican there is a golden, marbled mottle without particular bronzing or rustiness. None of the many sweet cherry trees inoculated with this disease has had leaves with any pronounced necrosis, such

as produced by the Severe Rusty Mottle described by Reeves (5, 6) and by the type of rusty mottle described from Utah by Rhoads (7, 8). Toward the end of summer, however, there may be slight necrosis following bronzing or rustiness. If this occurs it is usually in very small spots when compared with the large necrotic spots in leaves affected by Severe Rusty Mottle.

TABLE 1.—*The reactions of primary hosts to inoculation with the virus of Mild Rusty Mottle and the reactions of secondary hosts following inoculation from the primary host*

Source of virus, host inoculated, and reaction[a]		Source of virus, host inoculated, and reaction[a]	
From sweet cherry		From sour cherry	
To cherry		To cherry	
Napoleon	+	Napoleon	+
Bing	+	Bing	+
Black Republican	+	Black Republican	+
Lambert	+	Lambert	+
Mahaleb	−	Mazzard	+
Mazzard	−	Montmorency	+
Montmorency	+	To Bing cherry	+
Kwanzan	−	Amanogawa	−
To Bing cherry	−	Kwanzan	−
To Shirofugen cherry	−	Naden	−
Mt. Fuji	−	Shirofugen	−
Shirofugen	−	To peach	
Golden	+	Elberta	−
Deacon	−	J. H. Hale	−
Schmidt	+	Early Muir	−
To Prunus virginiana	−	To Rio Oso Gem peach	−
To peach		To Bing cherry	+
Elberta	−	To Black Republican cherry	+
Early Muir	−	Rio Oso Gem	−
To Bing cherry	+	To Bing cherry	+
To Black Republican cherry	+	To Italian prune	−
To Rio Oso Gem peach	−	To Bing cherry	+
To Coates plum	−		
To Burbank plum	−		
To French prune	−		
J. H. Hale	−		
To Early Muir peach	−		
To J. H. Hale peach	−		
To Bing cherry	−		
Rio Oso Gem	−		
Rochester	−		
To Italian prune	−		
To Bing cherry	+		

[a] The plus sign (+) indicates symptoms of Rusty Mottle in leaves. The minus sign (−) indicates the absence of symptoms. Some hosts on which symptoms were lacking acted as carriers of the virus.

In flowering cherries. Among the flowering cherries (*Prunus serrulata*) inoculated with Mild Rusty Mottle, only the Kwanzan and Naden varieties have shown any mottle with rustiness in the leaves, while the Amanogawa, Mt. Fuji, and Shirofugen varieties have had no leafy symptoms. The Mild-Rusty-Mottle symptoms in leaves of Kwanzan and Naden are, however, quite sparsely scattered over the tree or limited to certain areas of the tree, such as in single branches. In some instances even Kwanzan and Naden trees showed no symptoms, although infected with the virus. Latent viruses in·

sweet cherries, of course, have always complicated the study of any other sweet-cherry viruses in flowering cherry varieties (4).

In sour cherries. The Mild-Rusty-Mottle virus was found to occur in Montmorency cherry naturally, and it was transmitted to cherries, peaches, and prunes (Table 1). The leaf symptoms in Montmorency cherry artificially inoculated with Mild Rusty Mottle from either sour or sweet cherry were essentially the same as those observed in Montmorency trees naturally infected. The leaves in which vein necrosis and the resulting bronzing appear in early season, drop later, and no leaf symptoms may be seen during the remainder of the season. No die-back has occurred in any of these trees, which were inoculated three years ago.

In seedling cherries. In general, inoculated Mazzard seedlings show the same symptoms of Mild Rusty Mottle as do standard varieties of sweet cherry; however, there is some difference in individual Mazzard trees. Some have more of the reddish bronzed coloration in the leaves while others have definite ring spots with bronzed margins, and some show a finely stippled mottle.

Mahaleb seedlings have shown no leaf symptoms of the disease, but they may be symptomless carriers.

In wild hosts tested. *Prunus virginiana* (Eastern Chokecherry) and *Osmaronia cerasiformis* (Indian Plum) were not affected by inoculations, but no attempt was made to recover the virus from inoculated plants.

In peaches. The study of Mild Rusty Mottle on peach trees has been difficult, because all sources of inoculum have also included one or more ring-spot (2) or other sweet-cherry latent viruses (4) in addition to the Mild-Rusty-Mottle virus. When such buds with this mixed inoculum were grafted into peach trees, the latter became severely dwarfed and rosetted, with thickened twigs and short internodes. The leaves were smaller and darker green than normal and without mottling. Somewhat similar symptoms have been observed in peach trees inoculated with sweet or sour cherry having only latent or ring-spot viruses.

Nearly 100 peach trees of several varieties were inoculated from infected sour and sweet cherry, but in no case has there been any symptom which could be attributed to Mild Rusty Mottle alone. Mild-Rusty-Mottle virus was recovered from inoculated peach trees. These are reported in table 1.

In prunes. When the disease was inoculated into prune and plum trees of several varieties, no leaf symptoms resulted. Italian prune was proved to be a carrier of the virus by transfer back to Bing cherry, but the other varieties have not been so tested.

SYMPTOMLESS CARRIERS

As may be observed in table 1, J. H. Hale, Early Muir, Rio Oso Gem peach, and Italian prune have been proved, by return inoculation to sweet cherry, to be symptomless carries of Mild-Rusty-Mottle virus. The majority of stone fruit varieties which do not show symptoms may prove to be symptomless carriers.

COMPARISON OF MILD RUSTY MOTTLE AND SEVERE RUSTY MOTTLE

These two diseases are distinct in several respects. These differences are reflected particularly by the degree of severity on trees, and in different expressions of symptoms in leaves, fruit, bark, and growth of branches.

The decline of old sweet cherry trees infected with Mild Rusty Mottle is apparently not nearly so rapid as that brought about by Severe Rusty Mottle. The fruits of sweet cherry trees infected with Mild Rusty Mottle are normal in size and quality, whereas those of trees of the same varieties affected by Severe Rusty Mottle are insipid and reduced in size. The roughened bark, mentioned by Rhoads (7) has never been observed in trees infected with Mild Rusty Mottle; however, three Bing trees inoculated with the type of Severe Rusty Mottle from Washington developed a superficial splitting of the bark on one-year-old wood.

TABLE 2.—*The reactions of primary hosts to inoculation with the virus of Severe Rusty Mottle and the reactions of secondary hosts following inoculation from the primary host*

Source of virus, host inoculated, and reaction[a]		Source of virus, host inoculated, and reaction[a]	
From sweet cherry		From Early Muir peach	
To Early Muir peach	+	To Nonpariel almond	−
Montmorency cherry	+	To Tilton apricot	+
Bing cherry	+	To Kwanzan cherry	+
To Amanogawa cherry	+	To Shirofugen cherry	+
To Kwanzan cherry	+	To Early Muir peach	+
To Mt. Fuji cherry	+	To J. H. Hale peach	+
To Naden cherry	+	To Rio Oso Gem Peach	+
To Lovell seedling peach	+	To Rochester peach	+
To Prunus virginiana	+	To Burbank plum	−
		To Coates plum	−
		To French prune	−

[a] The plus sign (+) indicates symptoms of Rusty Mottle in leaves. The minus sign (−) indicates the absence of symptoms.

Leaf casting of Bing cherry trees affected with Severe Rusty Mottle is usually sufficient to cause the trees to have a stripped, bare, or defoliated appearance (6). Such severe leaf cast has never been observed in trees infected with Mild Rusty Mottle; although there may be some leaf cast in early summer, the trees do not appear noticeably defoliated.

Severe necrosis of the chlorotic areas in leaves of sweet cherry trees infected with Severe Rusty Mottle is usually apparent by late spring or early summer, while in trees affected by Mild Rusty Mottle a necrosis other than bronzing is seldom, if ever, seen as a leaf symptom.

After about the first of June the leaf symptoms of Severe Rusty Mottle show on the leaves almost to the tips of the branches, while in Mild Rusty Mottle there are no leaf symptoms for some distance back from the tips. The leaf symptoms of sweet cherries infected with either of these diseases seem not to be influenced by changes in temperature, but hold throughout the season.

In certain hosts, such as peach varieties, chokecherry (*Prunus virgin-iana*), and some varieties of flowering cherry (*P. serrulata*), inoculated with Mild Rusty Mottle, there are no particular leaf symptoms, whereas the same varieties and species inoculated with Severe Rusty Mottle show the rustiness and bronzing in the leaves similar to that described in sweet cherries. (Compare tables 1 and 2.) In peaches the leaf symptoms of Severe Rusty Mottle start as a chlorotic mottling in circular areas with diffuse edges. The leaves gradually turn yellow to rusty orange with greenish islands. The earliest symptoms are sometimes quite like asteroid spot (1) and then go through much the same series of symptom development as in sweet cherries, except that there is no necrosis. Since this Severe-Rusty-Mottle inoculum proved to be free of sweet-cherry latent viruses, the symptoms described are apparently those of Severe Rusty Mottle. Tilton apricot gave the same symptoms as peach when inoculated with Severe Rusty Mottle. Flowering cherries react in much the same way as peaches and apricots.

It would perhaps be well to point out specifically the difference between Mild Rusty Mottle as it occurs in Oregon and Washington and the form which Rhoads has described in Utah (7, 8). Rhoads' suggestion that "even the meager occurrence of the necrotic spots associated with this disease constitutes a very reliable means of detecting it and that a diagnosis made on this basis will err mainly in being too conservative in some orchards," does not apply to Mild Rusty Mottle in the least. The Utah strain, however, may be a form of Severe Rusty Mottle.

NOMENCLATURE

Since there are so many differences between Mild and Severe Rusty. Mottle there is considerable question as to the relation between the two. There seems to be but one characteristic in common, namely, the rustiness in the leaf symptoms in sweet cherry. This at least should be considered generic. But too little is known of the two forms to decide which is typical or which might be a variety of the other. Surely in most cases a plant is given specific rank if it differs in three or more characters from its nearest congener. In this case there are six or seven character differences, but at present we merely propose Mild Rusty Mottle as a common name and Severe Rusty Mottle as a common name of the form Reeves has described.

CONTROL

As is true with other diseases of this nature, the control in future orchards rests in the use of clean nursery stock. Where healthy trees are to be top-worked to other varieties great care should be exercised in the selection of budwood free of harmful viruses. In the established orchard where few trees are infected, the diseased trees should be removed at once. In cases where the removal of infected trees will too greatly deplete the established orchard, they may be allowed to remain until unprofitable. Where a thinning program is necessary, mapping of the diseased trees should precede

pulling, and the pulling of trees be so arranged as to remove the greater number of diseased trees.

SUMMARY

Mild Rusty Mottle is described as distinct from the severe form described by Reeves in 1940. It occurs in eastern and western Oregon, and in parts of Idaho and Washington. Trees affected with it persist for a number of years, but apparently are more subject to winter injury and drouth than healthy trees. The leaves show a rusty and bronzed mottle, but without the necrosis so prevalent in the severe form.

The disease has been found natively in sweet and sour cherry trees, and by graft inoculations has been transferred to sweet and sour cherry, to peach, to flowering cherry, and to Italian prune. The symptoms in various hosts are described. Symptomless carriers of the disease are all of the varieties of peaches tested and Italian prune. Infected Montmorency cherry is symptomless for most of the growing season. The differences between the mild and severe forms of rusty mottle are pointed out. Since these two viroses are so distinct, the common names, Mild Rusty Mottle and Severe Rusty Mottle, are proposed.

Control is through the use of clean scion wood in the nurseries and roguing out affected trees in orchards.

OREGON AGRICULTURAL EXPERIMENT STATION,
CORVALLIS, OREGON.

LITERATURE CITED

1. COCHRANE, L. C., and C. O. SMITH. Asteroid spot, a new virosis of the p . Phytopath. 28: 278–281. 1938.
2. ————, and L. M. HUTCHINS. A severe ring-spot virosis of pe (Abstr.) Phytopath. 31: 860. 1941.
3. COE, DONALD M. Report of the 1942 stone fruit virus disease survey i Washington. Washington State Dept. Bul. 2. 1943.
4. MILBRATH, J. A., and S. M. ZELLER. Latent viruses in stone fruits Science 101: 114–115. 1945.
5. REEVES, E. L. Rusty Mottle, a new virosis of cherry. (Abstr.) Phytopath. 30: 789. 1940.
6. ————. Virus diseases of fruit trees in Washington. Washington State Dept. Agr. Bul. 1. 1943.
7. RHOADS, ARTHUR S. Virus and virus-like diseases of sweet cherry in Utah, and notes on some conditions affecting various fruit crops. U. S. Dept. Agr., Pl. Dis. Reptr. 29: 5–17. 1945.
8. ————. Symptom expression of Rusty Mottle in Utah sweet cherry orchards. U. S. Dept. Agr., Pl. Dis. Reptr. 29: 613–614. 1945.
9. ZELLER, S. M. Virus diseases of stone fruits. Oregon State Hort. Soc., Ann. Rept. 34: 83–90. 1942.

FIELD METHODS OF TESTING FOR ROOT-KNOT INFESTATION[1]

A. L. SMITH AND A. L. TAYLOR

The testing of crop varieties for farm use, the breeding of crops for root-knot resistance, the evaluation of the root-knot infestations in soils and the comparison of different crops for resistance require a precise method of measuring the degree of infestation by the root-knot nematode, *Heterodera marioni* (Cornu) Goodey. Systems used in the past have generally lacked a simple method of expressing the results numerically and were, therefore, not subject to statistical evaluation. Accordingly, investigations were directed toward establishing a satisfactory means of expressing the degree of root-knot infestation obtained in field experiments.

Godfrey (5) reviewed the earlier literature on attempts to arrive at soil populations of the root-knot nematode, first by actual counting and identifying of nematodes in a soil sample, and later by root-gall counts on indicator crops. His studies with indicator crops showed a high correlation between the percentage of infested plants and the average number of galls per plant when such counts were low, averaging 1 to 30 per plant. The gall count and percentage count data were transformed by him to estimates of actual soil populations. At the present time the gall-count system is used extensively in detailed studies; but for field use, involving large numbers of plants, it is too slow and expensive. The rapid grouping system of McKinney (9), based on grouping of plants in classes depending on magnitude of disease, has become more widely used for handling field experiments. Guba (6) reported percentages of indicator plants with zero, light, moderate, and severe infection. Barrons (2) used 5 classes in studying root-knot resistance in bean and pea seedlings. His categories were: class 1, not infested; and classes 2 to 5 with increasing amounts of galls. At the beginning of the study standards representing each class were selected for comparing the remainder of the roots. Smith (11), employing 5 classes, graded cotton roots on a basis of percentages of the total root system showing galls. Newhall and Stark (10), Kincaid and Reeves (8), and Stark *et al.* (12) report work with root knot using several modifications of the grouping system.

The determination of resistance to infestation involves a consideration of the factors affecting the production of disease symptoms. Where the root-knot nematode is concerned, plants have all degrees of resistance ranging from immunity to very high susceptibility. Apparently root-knot-resistant plants are not resistant because of any ability to prevent the entrance of

larvae from the soil (3), but rather through the lack of ability of the larvae to grow to maturity within the roots. In a highly resistant plant, such as *Crotalaria spectabilis*, larvae enter and even form small and inconspicuous knots, but never develop to maturity (1). The same condition was noted by Christie and Albin (4) for several other species of plants tested against various strains of root-knot nematodes. With certain exceptions reported by Steiner (13), notably freesia and cyclamen, root-knot nematodes form more or less conspicuous knots on the roots of host plants. Since the degree of infestation of a given plant which has been in the soil for several months will be somewhat related to the number of progeny produced by the original invaders of the plant, variability in egg production is a factor of resistance. If few or many eggs are produced, the end result will be a plant lightly or heavily infested, respectively. It seemed apparent that resistance in typical cases could be evaluated by observing the gall formation on plants which had been grown to maturity (or near maturity) in soil heavily infested with root-knot nematodes.

DIFFERENTIATION OF VARIETIES

The differentiation of crop varieties in regard to root-knot resistance was studied, using soybeans and cotton. Four experiments with soybeans and four with cotton were conducted over a 3-year period, 1941 to 1943. The tests were at Tifton, Plains, and Perry, Georgia. At Tifton, one experiment with soybeans was on soil artificially contaminated with root-knot nematodes by burying infested roots. The remainder of the tests were on naturally contaminated soils. Seed from the same lot of soybeans were used in all tests. The cotton seed were from different lots in different years. Replications consisted of single-row, randomized plots long enough to contain approximately 100 plants. Soybeans were planted the first week of May and cotton the first week in April. These are approximately the normal planting dates for both crops. Soil temperatures in April are somewhat low for normal nematode activity but are more favorable in May at the time of planting soybeans.

The plants were allowed to grow to maturity (September and October) in the various experiments to permit the recording of yield data, then dug and examined. In digging, an effort was made to remove a large proportion of the root system, but no effort was made to get all of it. The roots of all plants were examined by visual inspection for the presence of root-knot galls, and each plant was classified according to degree of infestation. The categories were similar to those used by Smith (11); Class 0 = all roots without visible galls, Class 1 = 1 to 25 per cent of roots with galls, Class 2 = 26 to 50 per cent, Class 3 = 51 to 75 per cent and Class 4 = 76 to 100 per cent of roots with galls. This system was used for recording data obtained from all tests at Plains and Perry. Similar infestation categories were used for data obtained from the Tifton tests except that arbitrary standards were set up for each class as reported by Barrons (2). Noninfested plants were

placed in Class 0, those with light infestation in Class 1, those with very heavy infestation in Class 4, and those with intermediate degrees of infestation in Classes 2 and 3.

The two systems are summarized in table 1.

Data were calculated in all instances by the McKinney (9) formula as modified by Horsfall and Heuberger (7) who applied it to a defoliation disease of tomatoes. It is as follows:

$$\text{Disease index} = \frac{\Sigma \text{ category numbers}}{\text{No. plants} \times 4} \times 100$$

The summarization of category numbers is obtained by multiplying the number of plants in each category by their respective category numbers from 0 to 4, and adding the products.

It is proposed that "root-knot index" apply to infestation data obtained by the first of the two methods, using the above formula. Thus the "root-

TABLE 1.—*Categories established for the determination of root knot by the root-knot index system and by the relative index system*

Infestation category	Root-knot index system[a]	Relative index system[b]
0	No infestation	No infestation
1	1 to 25 per cent	Light
2	26 to 50 per cent	Medium
3	51 to 75 per cent	Heavy
4	76 to 100 per cent	Heavy

[a] Used in tests at Plains and Perry.
[b] Used in tests at Tifton.

knot index" becomes an estimate of percentage infestation with a range from zero to 100 and comparable to other disease data based on percentage of infection. To indicate the index obtained by the second method as used in the Tifton experiments it is suggested that "relative index" or "relative root-knot index" be applied. By this latter system the indexes are relative only to others in that specific study since new standards are adopted for each experiment.

RESULTS OF SOYBEAN VARIETY TESTS

Root-knot indexes, relative root-knot indexes, and least significant mean differences for the soybean variety tests are given in table 2. Examination of the data indicates three distinct resistance groups are represented by the five varieties. Laredo was more resistant in all cases, Biloxi and Otootan were intermediate, and Clemson and Georgian were most susceptible. Only the 1942 Tifton test gave clear-cut separation of all three groups. In the 1941 Tifton experiment, on artificially infested soil, the Clemson variety was in the group with Biloxi and Otootan. The two 1941 field tests likewise have one variety each which is not clearly differentiated. The 1942 test at Tifton, with a lower requirement for significant differences, segregates the varieties more fully than the remainder of the studies. This may be attrib-

uted largely to the use of 6 replications as compared with 4 or 5 replications in the other tests, and a more uniform distribution of nematodes in the soil. Field infestations of root-knot nematode are generally spotted and even with small tests the results suggest a minimum of 6 replications may be necessary for most locations.

The method of recording data did not affect the conclusions that might be drawn from individual tests. The root-knot index system used in the

TABLE 2.—*Root-knot indexes and relative indexes of soybean varieties, 1941–1942*

Variety	Series						Varietal mean
	1	2	3	4	5	6	
Artificially infested soil--Tifton, 1941[a]							
Laredo	21	19	25	28			23.2
Biloxi	59	43	36	55			48.2
Otootan	30	39	78	57			51.0
Clemson	66	44	49	79			59.5
Georgian	68	84	86	94			83.0
L.S.M.D. (0.05)							20.0
Naturally infested soil—Tifton, 1941[a]							
Laredo	3	5	25	8	9		10.0
Biloxi	32	20	18	15	48		26.6
Otootan	27	23	14	27	40		26.2
Clemson	36	36	19	51	70		42.4
Georgian	49	66	54	26	56		50.2
L.S.M.D. (0.05)							16.6
Naturally infested soil—Plains, 1941[b]							
Laredo	8	26	21	5	2		12.4
Biloxi	27	51	59	34	42		42.6
Otootan	46	89	62	39	29		53.0
Clemson	96	100	99	76	18		77.8
Georgian	94	52	92	80	63		76.2
L.S.M.D. (0.05)							24.5
Naturally infested soil—Tifton, 1942[a]							
Laredo	9	12	6	13	6	9	9.1
Biloxi	30	20	17	19	22	40	24.7
Otootan	27	25	34	20	37	51	32.3
Clemson	30	58	50	39	54	41	45.3
Georgian	57	46	61	37	37	65	50.5
L.S.M.D. (0.05)							11.0

[a] Relative root-knot indexes.　　　[b] Root-knot indexes.

Plains test resulted in a wider spread between the most susceptible and most resistant variety than was found by the relative index system used in the two Tifton tests on naturally infested soils. A wider spread would be of value in differentiating a large number of entries. However, the relative index spread obtained by using a set of standards is affected by the standards selected. With added experience in the adoption of standards the full index range might be utilized.

RESULTS OF COTTON VARIETY TESTS

The cotton variety tests were conducted in a manner similar to the soybean tests. Six replications were used, except at Perry where data were recorded from only three. The "index" was obtained at the Plains and Perry locations and "relative index" at Tifton. The results (Table 3) indicate Coker 4 in 1-5 to be the only variety with outstanding resistance. Rhyne's Cook was significantly more resistant than Stoneville 2B in the 1942 Plains and Tifton tests and also more resistant than Coker 100 Wilt-1 in the Tifton test. Early Wilt has lower index numbers than several varieties in the 1941 and 1942 tests but was significantly more resistant than other varieties in only one study, the 1942 Plains test. Differences among varieties in the 1943 Perry test were found nonsignificant and the data are shown only to emphasize the importance of utilizing sufficient replications in the presence of such large inter-plot variability.

TABLE 3.—*Root-knot indexes of cotton varieties*

Variety	Plains, 1941	Plains, 1942	Tifton,[a] 1942	Perry, 1943
	Index	*Index*	*Index*	*Index*
Coker 4 in 1-5	62.0*[b]	59.7*	24.3*	47.7
Rhyne Cook	87.5	86.3*	40.8*	57.0
Early Wilt	91.3	83.0*	50.0	36.0
Station 21 .. .	97.8	94.8	52.0	63.3
Stoneville 2B	93.5	98.2	56.0	73.7
Coker 100 Wilt-1 .	94.7	93.5	56.5	61.3
L.S.M.D. (0.05)	12.3	10.8	13.9	c

[a] Tifton data are relative indexes.
[b] Starred (*) figures indicate that a variety was significantly more resistant than one or more other varieties.
c Differences not significant.

This group of varieties may be considered as representing the range in root-knot resistance in upland cotton varieties commonly planted in the Southeast (11). All, except Coker 4 in 1-5, are relatively susceptible and they tend to group at the upper range of susceptibility as illustrated in the Plains 1941 test in which 4 of 6 varieties have indexes varying from 91.3 to 97.8. The same 4 varieties have relative indexes varying from 50.0 to 56.5 in the Tifton 1942 test. In these studies with cotton varieties there appears to be no preference between the two methods used for recording data. However, minor differences in gall size were noted in the 4 most susceptible varieties and in order to utilize these differences the relative index may be preferable. Relative index utilizes both size and number of galls in evaluating susceptibility, whereas, the root-knot index is based on numbers and largely disregards size of galls.

ROOT-KNOT RESISTANCE OF DIFFERENT CROPS

A number of crops grown in rotation with cotton were planted at Plains, April 10, in a soil where root knot of cotton had previously occurred. The

TABLE 4.—*Root-knot indexes of several different interplanted crops.* **Plains, Georgia, 1945**

Crop	June 29	July 21	October 20
	Index	*Index*	*Index*
Cotton (Deltapine)	37.9	55.7	100
Corn (Whatley's)	77.1	75.8	Mature, roots decayed
Sorghum (Texas Seeded Ribbon)	9.5	30.2	Older roots decayed
Soybean (Mamloxi)	35.5	34.1	No apparent increase
Soybean (Biloxi)	21.9	16.0	do
Peas (Brown Crowder)	100.0	86.2	Killed by root knot, Aug. 1 to 20
Lespedeza (Korean) .	71.5	95.2	do Aug. 15 to 30
Lespedeza (Kobe)	44.8	68.0	do Sept. 15 to 30
Vetch (Hairy)	100.0	100.0	All plants dead July 1
Velvet bean .. .	7.5	0.0	0.0

test consisted of single-row plots, 50 feet long, with 4 replications. Parts of each plot were dug on June 29 and July 21 with the remainder being left for final observation on October 20. The development of the infestation (Table 4) could be followed by the root-knot indexes measured on different dates. Brown Crowder peas, Kobe and Korean lespedeza were very susceptible and died before maturity and seed production. Hairy vetch was the most susceptible crop tested, although it was not apparent whether the killing was due to root knot or partially due to hot weather. With soybeans there was no tendency for rapid increase of root knot as the season progressed but with the rather tolerant cotton, infestation continued to increase to the end of the season. Velvet beans were lightly infested early. In later diggings, due to the vigorous, deep-growing root system, few small roots were found and galls were not observed. Fibrous-rooted crops such as corn and sorghum are quite tolerant to root knot. The greater resistance of sorghum is illustrated by the lower index. Older roots of sorghum were decaying from fungal attacks and were continuously replaced by newer ones, and final data on root knot were not obtained.

CONTROL STUDIES WITH NEMATOCIDES

Use of the root-knot index system of recording infestation data in soil-fumigation studies is illustrated in table 5. This study was conducted in

TABLE 5.—*The effect of different rates of application of D-D and chloropicrin on root-knot infestation in tomatoes.* *Plains, Georgia, 1944*

Nematocide	Pounds per acre	Spacing of injection points	Amount injected	Root-knot index
		Inches	*Ml.*	
D-D	192	36 × 18	7.5	41.1
D-D	256	36 × 18	10.0	21.8
D-D	385	18 × 18	7.5	10.3
D-D ....….........	512	18 × 18	10.0	0.0
Chloropicrin	535	18 × 12	5.0	22.2
Check	....	....	....	84.7
L.S.M.D. (0.05)	....	....	....	25.0

cooperation with the "Experiment Station Committee" for investigation of new nematocides. The soil treatments were applied April 25 and data were recorded October 26, 1944. Marglobe tomato plants were used as the test crop. The root-knot indexes illustrate the control and lasting effects of different rates of application of D-D[2] in comparison with chloropicrin. Since the maximum number of plants per plot was 6 in this test the requirement for significance is relatively higher than might have been obtained with larger populations.

DISCUSSION

A comparison of the "root-knot index" and "relative index" systems for determining varietal resistance gave essentially similar results from location to location, from year to year, and by different workers. Under conditions of the studies conducted neither system was preferable to the other but there was some observational evidence that where larger numbers of crop varieties are being tested under conditions of severe infestation the relative index may be preferable. The index system otherwise permits a closer comparison between different studies since it is based on percentage infection, whereas, arbitrary standards are utilized for the relative index.

Data collected at Plains and Tifton were generally in good agreement, indicating that there is little difference between the host preferences of the root-knot nematodes at those two locations. It should be emphasized that, in the light of the data presented by Christie and Albin (4), this might not be true for other locations. Of 13 nematode populations tested by them, only one gave a heavy infestation on cotton, one gave a light infestation, two a very light infestation, one no infestation, and the other eight gave only a trace of infestation with no eggs produced. On Laredo soybeans 10 populations were tested by Christie and Albin (4) and 2 of the nematode populations failed to infest at all, 2 more had only a trace of infestation with no eggs, 3 had a very light infestation, and 3 a light infestation which might correspond to that found at Plains and Tifton in the study reported here. Until more is learned concerning the number of races of root-knot nematode and their locations, data on resistance of hosts of the root-knot nematode should be considered as applying only to the locations specified, and not as being of general significance. On the other hand, continued testing of resistance, if such a plan as the one here presented is followed, will provide the data for conclusions as to the habits and locations of the various races of root-knot nematodes. It would also provide information of great practical value for the planning of rotations.

The lack of uniform method of recording infestation data in relation to studies on soil fumigation is illustrated in cooperative reports and other recent publications (8, 10, and 12). Studies on a regional basis, involving a wide range of infestation intensity with the accompanying need for com-

---

[2] D-D is a mixture of dichloropropylene and dichloropropane containing a small percentage of more highly chlorinated compounds.

parison and integrating with related experiments, probably may best be evaluated by use of the root-knot index rather than the relative index.

Experiments reported here were not designed to answer the question concerning number of replications or number of plants necessary to obtain the results desired; however, some information was obtained. With crop varieties the number of replications used varied from 3 to 6. The evidence indicates that, due primarily to the extreme variations in root-knot larvae populations in the soil, 6 replications are preferable to a smaller number. Three replications were not sufficient to obtain significant differences between any of the varieties at the Perry location. In the studies reported here the number of plants per plot used for determining varietal differences was arbitrarily placed at 100. This number is perhaps greater than necessary, 40 or 50 in most instances would give a satisfactory plot reading. For the studies with nematocides 4 to 6 plants per treatment gave significant differences between rates of applications of D-D. However, larger numbers of plants are undoubtedly necessary for more exact information. Godfrey (5) concluded that from 25 to 100 indicator plants per block were sufficient to give a satisfactory estimate of the root-knot infestation, the accuracy of estimates being greater, of course, with the larger numbers.

SUMMARY

A system for testing root-knot resistance of plant varieties in the field has been developed. At least 6 replicated plots for each variety are located on soil known from previous examination to have a heavy infestation of root-knot nematodes. Plantings are made when soil temperatures are favorable for activity of the nematodes and the plants are allowed to grow to maturity. Roots are then removed, examined, and grouped, using the McKinney system. Disease indexes are then calculated, using the Horsfall and Heuberger modification of the McKinney system.

Two systems of determining root-knot infestation grades were compared for differentiating soybeans and cotton: one, based on the percentage of the root system with visible galls; the second, based on the adoption of standards for each root-knot class with the remainder of the roots graded by comparison with the standards selected. It is proposed that "root-knot index" be used to designate infestation indexes obtained by the percentage method and "relative root-knot index" used for designating infestation indexes obtained by comparison with adopted standards. The two systems were about equally effective in differentiating varieties. The consistency of the results from year to year and location to location indicates the validity of both methods.

Root-knot index data were obtained on several interplanted crops for evaluating their possibilities in a root-knot-reducing rotation. The seasonal development of infestation was demonstrated and the crops segregated for root-knot reaction.

Possibilities for use of the root-knot-index were demonstrated in relation to studies on chemical control.

DIVISION OF COTTON AND OTHER FIBER CROPS AND DISEASES
DIVISION OF NEMATOLOGY
    U. S. DEPARTMENT OF AGRICULTURE, AND
GEORGIA AND COASTAL PLAINS EXPERIMENT STATIONS,
    EXPERIMENT AND TIFTON, GEORGIA.

LITERATURE CITED

1. ANONYMOUS. Control of root knot by the use of Crotalaria spectabilis. 23rd Ann. Rept. Ga. Coastal Plain Exp. Sta. Bul. 36: 112–113. 1942–1943.
2. BARRONS, KEITH C. A method of determining root-knot resistance in beans and cowpeas in the seedling stage. Jour. Agr. Res. [U.S.] 57: 363–370. 1938.
3. ———————. Studies of the nature of root-knot resistance. Jour. Agr. Res. [U.S.] 58: 263–271. 1939.
4. CHRISTIE, JESSE R., and FLORENCE E. ALBIN. Host-parasite relationships of the root-knot nematode Heterodera marioni. I. The question of races. Proc. Helminth. Soc. Wash. 11: 31–37. 1944.
5. GODFREY, G. H. Indicator plants for measuring soil populations of the root-knot nematode, Heterodera marioni (Cornu) Goodey. Soil Sci. 38: 3–27. 1934.
6. GUBA, E. F. Carbon disulphide emulsion for the control of the root-knot nematode. Mass. Agr. Exp. Sta. Bul. 292. 1932.
7. HORSFALL, JAMES G., and JOHN W. HEUBERGER. Measuring magnitude of a defoliation disease of tomatoes. Phytopath. 32: 226–232. 1942.
8. KINCAID, RANDALL R., and JESSE REEVES. Cultural practices for root-knot control between annual crops of cigar-wrapper tobacco. Fla. Agr. Exp. Sta. Bul. 392. 1943.
9. MCKINNEY, H. H. Influence of soil temperature and moisture on infection of wheat seedlings by Helminthosporium sativum. Jour. Agr. Res. [U.S.] 26: 195–217. 1923.
10. NEWHALL, A. G., and F. L. STARK, JR. Chloropicrin and ethylene dichloride for root-knot nematode control. Phytopath. 32: 626–630. 1942.
11. SMITH, A. L. The reaction of cotton varieties to Fusarium wilt and root-knot nematode. Phytopath. 31: 1099–1107. 1941.
12. STARK, FRANK L., JR., BERT LEAR, and A. G. NEWHALL. Comparison of soil fumigants for the control of the root knot nematode. Phytopath. 34: 954–965. 1944.
13. STEINER, G. Tylenchus pratensis and various other nemas attacking plants. Jour. Agr. Res. [U.S.] 35: 961–981. 1927.

FACTORS AFFECTING INFECTIVITY, SPREAD, AND PERSISTENCE OF PIRICULARIA ORYZAE CAV.[1]

AXEL L. ANDERSEN, B. W. HENRY, AND E. C. TULLIS[2]

INTRODUCTION

Piricularia oryzae Cav., the cause of the "blast" disease of rice, is found in nearly all the rice-growing regions of the world. In the United States it is found principally in the rice-growing areas of Arkansas, where it annually causes some damage to the Japanese-type rice varieties.

The effect of various environmental factors upon the infection of rice by *Piricularia oryzae* has been considered by several investigators. Hemmi (8) and Suzuki (17, 18) found that plants grown in dry soils were more susceptible to infection than plants grown in wet soils. Tochinai and Komuja (21) found that a 3-day exposure to drought prior to inoculation was sufficient to increase the amount of infection and that an exposure for an equal length of time to a saturated atmosphere resulted in reduced infection. It was shown by Abe (2) that rice seedlings were most susceptible to infection when grown at low soil temperatures (20° C.) and least susceptible when grown at higher soil temperatures (28–32° C.). Several authors (14, 18, 20) found that susceptibility increased as the amount of nitrogenous fertilizer was increased. Abe (1) demonstrated that infection was more intense when seedlings were kept shaded or in darkness. Imura (12) studied the development of lesions on seedlings kept under various degrees of light intensity. He found that the initial development of the lesions occurred most rapidly on those plants which were kept slightly shaded but that the maximum enlargement finally occurred on those given the most light. Sakamoto (13) found a marked increase in infection after the plants were exposed to strong winds. This was attributed to wounds on the leaves due to rubbing or possible physiological changes in the leaf tissue due to drying. Tochinai and Komuja (21) also found that plants exposed to injury were more susceptible. According to Abe (3), no infection takes place at relative humidities below 90 per cent because of the failure of spores to germinate. Hemmi and Imura (11) found that air humidities in excess of 89 per cent were necessary for conidium formation. Hemmi and Abe (9) found that air temperatures from 24° to 28° C. favored the rapid development of the initial stages of leaf infection and, at these temperatures, a minimal period of from 6 to 8 hours of continuous wetting of the plants was necessary to establish infection.

Investigations reported in this paper deal with varietal resistance, effect of age of plant on infection, environmental factors affecting primary and secondary infection, longevity of conidia, reduction of disease development by *Helminthosporium oryzae* van Breda de Haan, and susceptibility of other cereals. The field experiments were at Beaumont, Texas.

MATERIALS AND METHODS

Source and Production of Inoculum

Two field isolates of *Piricularia oryzae* were obtained from Arkansas. The one was isolated from dead rice stubble in 1943 and the other from Cody rice in 1944. Both of these isolates, and re-isolates obtained by their passage through the host, were used in the present study.

The conidia used for inoculation of the host were produced on a grain substrate containing equal parts by weight of Hegari sorghum and oats. A satisfactory method for preparing the substrate was to add 100 g. of each grain and 230 ml. of water to a 2800-ml. Fernbach flask. The grain was presoaked for 2 hours and then autoclaved for 20 min. at 15 lb. pressure on each of 3 successive days to insure complete sterilization. The substrate was seeded with a 10-ml. spore suspension obtained from stock cultures on 2 per cent rice-polish agar and containing at least 200,000 spores per ml. The seeded substrate was incubated at room temperature for 6 to 7 days and was aerated at a rate of 5 ml./min./g. of dry substrate for the entire period. The spores were then removed from the substrate by washing the whole in distilled water. The resulting suspension was used for inoculation of the host, or finely ground, black, neutral peat was incorporated with the suspension, the mixture filtered, the filter cake dried at 40° C. for 24 hours, powdered, and the resulting dust used as inoculum.

Inoculation of Plants

All the inoculations were made with the aid of hand-made dusters and atomizers (Fig. 1, B and C). The dusters were capable of dispensing from 1 to 10 g. of dust inoculum per min. with air pressure supplied by a hand atomizer bulb or a compressor. The atomizers had a dispensing capacity of 50 to 75 ml. of suspension per min. with air pressure applied by the compressor. Lower rates of application could be achieved by reducing the air pressure.

Considerable difficulty was encountered in securing uniform distribution of leaf infections on rice plants when the latter were inoculated with aqueous suspensions of conidia. The effectiveness of these suspensions was improved by the addition of 0.05 per cent sodium oleate and 0.25 per cent gelatin, making it possible to obtain a 4- to 10-fold increase in the number of leaf infections per plant and a more even distribution of leaf infection (5). Hence, in all cases where conidial suspensions were used for plant inoculations, the conidia were suspended in a sodium oleate-gelatin solution.

It was found that fresh conidia applied at a rate of 1 million in 10 ml. of suspension per 6-inch pot of 15 plants (3–5-leaf stage), provided sufficient inoculum for maximum infection. On an area basis, maximum infection occurred following the application of a conidial suspension applied at a rate of 1.0 million conidia per sq. ft.; and an application of 2.5 million conidia per sq. ft. was necessary if the inoculum was applied in the dry form using the duster. These rates were used throughout the experiments.
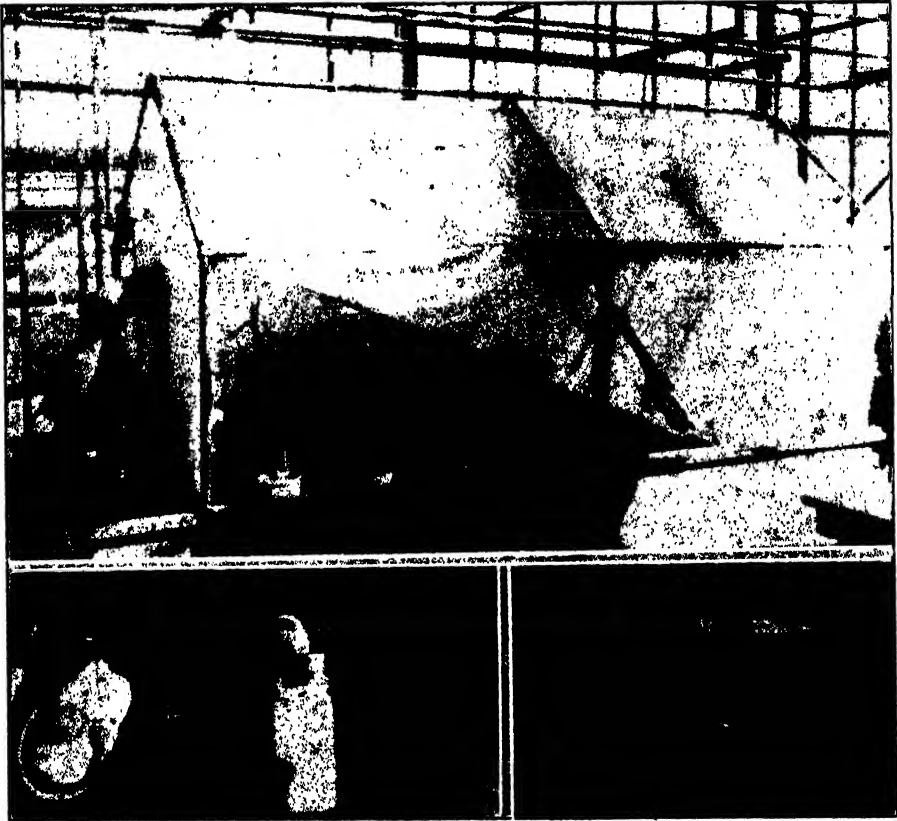

FIG. 1. A. Humidity tent containing rice plants in gallon and half-gallon glazed pots. Note overhead water spray in operation and Standard Humidifier Mod. 31 at end of tent. B. Duster used to apply dry conidial inoculum. C. Sprayer used to apply conidial suspension. The upper tube is attached to the compressor; the lower tube is inserted in the suspension.

Establishment of Infection

A humidity tent was used for providing favorable conditions for the establishment of *Piricularia oryzae* infection on host plants (Fig. 1, A). This tent consisted of a muslin cover supported by a steel frame over a deep, water-tight bench. An external overhead sprinkling system was used for keeping the muslin wet as an aid in maintaining humid conditions inside the tent. A water humidifier, set at one end of the bench, maintained a

high humidity within the tent so that the plants were covered with free moisture for as long a period as desired. Required temperatures (22–28° C.) were maintained in the tent by controlling the room temperature and the temperature of the water in the bottom of the bench. A constant record was maintained of the air temperature in the tent by means of a recording thermograph. Sufficient air circulation was produced by the humidifier to enable a study of secondary spread of *P. oryzae* under controlled conditions.

Evaluation of Results

The basic criteria, unless otherwise stated, for the evaluation of *Piricularia oryzae* infection on rice in the seedling (3–5-leaf) stage of development were (1) the number of leaf spots produced, (2) the number of leaves infected, and (3) the number of leaves killed. The death of individual plants was not used as a criterion since it rarely resulted from primary infection. It was only when the plants were subjected to repeated inoculations or placed under ideal environmental conditions for reinfection that they were actually killed by *P. oryzae*. The leaf lesion and infected leaf counts were made as soon as the spots could be distinguished and before they had begun to coalesce. This was generally from 4 to 9 days after inoculation. A count of the dead leaves was made approximately 2 weeks after inoculation.

HOST FACTORS RELATED TO INFECTION

Varietal Susceptibility

To insure rapid and conclusive results in greenhouse tests, several varieties of rice were required which were very susceptible to *Piricularia oryzae*. Seed of 17 Japanese short-grain and 2 American medium-grain varieties were secured and all were tested in the seedling (3–5-leaf) stage. Their relative susceptibility was estimated from (1) the rapidity of lesion development, (2) the size of the lesions, and (3) the killing of the leaves. On this basis the varieties were placed in 5 groups, as follows:

1. *Very susceptible:* Characterized by very rapid enlargement of lesions and killing of many of the leaves, with size of lesions not restricted. Onsen, Acadia, Wataribune, Caloro, Asahi, Wasa Shinriki, Colusa × Blue Rose, and Blue Rose.

2. *Susceptible:* Lesions enlarged more slowly than in group 1, resulting in the killing of some of the leaves, with size of lesions not restricted. Kinai 195, Hattan, and Guneki.

3. *Moderately susceptible:* Lesions large but definitely restricted, with only a small number of leaves killed. Haya Ozeki, Kameji, and Gin Bozu (C.I. 6355).

4. *Moderately resistant:* Lesion size restricted to 1–2 mm., no leaves killed. Akaho, Early Wataribune, Gin Bozu (C.I. 6873), and Meshibu.

5. *Resistant:* Infection readily established but with lesions remaining as small flecks on the leaves. Butte.

On the basis of this classification, and because of availability, Acadia and Onsen varieties were selected for greenhouse studies. The latter variety was favored because it was possible to secure seed free from *Helminthosporium oryzae*.

Stage of Plant Development

The age of the plant was an unreliable criterion of the stage of development of rice plants because of variation in the growth at various times of the year. Consequently, tests were carried out according to the stage of development of the plant as follows: (1) seedling, (2) tillering, (3) booting, (4) heading.

In order to have plants in various stages of development available at the same time, a series of Acadia and Onsen rice plantings was begun in the greenhouse in July, 1944, and new plantings were made at approximately 2-week intervals thereafter until the oldest plantings had reached the flower-

TABLE 1.—*Susceptibility of various parts of the rice plant at various stages of development*[a]

Tissues infected	Stage of plant development and age in weeks at time of inoculation			
	Seedling	Tillering	Late tillering and booting	Heading
	1–4	4–7	7–11	11–13
Leaves and leaf sheaths	X	X	Trace	0
Leaf and sheath junction	X	X	X	X
Nodes of panicle			X	X
Culm			0	X
Panicle[b]			X	X

[a] X = infection; 0 = no infection.
[b] Panicle infections include those of its branches, spikelets, lemma, and palea.

ing stage. All the plants were sprayed with an aqueous suspension of *Piricularia oryzae* conidia to determine their relative susceptibility to infection at the various stages (Table 1). It is apparent from these results that only plants in the seedling and tillering stages were susceptible to leaf and sheath infection, with the degree of resistance to these infections increasing as the plant became older. From the late tillering to the late booting stage the plants were very resistant to infection. It was not until they reached the late booting and heading stages that they again became susceptible to infection. During these later stages all the exposed parts of the panicle were subject to infection which resulted in "blasting." Completely blasted panicles resulted from infections of the nodes, culms, or entire heads, whereas partially blasted panicles resulted from infections of some of their individual parts.

Abe (4) studied the comparative susceptibility of the different portions of the rice plant to *Piricularia oryzae* and found that the greatest number

TABLE 2.—Percentage of blasted panicles and grains resulting from inoculations with Piricularia oryzae at various stages of development

Variety[a] and stage of development at time of inoculation	Noninoculated plants (control)				Inoculated plants			
	Panicles	Grains	Filled grains	Light grains	Panicles	Completely blasted panicles	Grains	Blasted grains[b]
			Pct.	Pct.				Pct.
Acadia								
Heading (flowering)	9	704	96	4	8	3	641	82
Early heading	10	740	89	11	10	9	810	93
Late booting-early heading	7	563	95	5	8	5	533	65
Booting	8	391	95	5	5	3	233	71
Onsen								
Flowering; soft dough	9	702	93	7	8	7	672	94
Booting; milk	6	627	91	9	7	7	499	100
Late tillering; flowering	9	631	90	10	9	5	536	82
Tillering; late booting and early heading	6	400	86	14	5	3	336	93

[a] Acadia variety was inoculated once. Onsen variety received 2 inoculations at 2-week intervals.
[b] Includes those grains which ordinarily would be light. (Note percentage of light grains in control.)

of leaf lesions, on plants in the 3–5-leaf stage, occurred on leaves "of medium size," and that the lesions on the youngest or oldest leaves were relatively few. He also noted that the number of diseased spots per unit length of leaf was greatest at the middle part. On the other hand, the number of lesions per unit length was greater near the tip than at the basal part of the young and medium leaves. On the older leaves the opposite relationship was observed. He concluded that "the most important factors which control distribution of the diseased spots on leaves seem to be a tendency of leaves to get wet (Benetzbarkheit), the angle of leaves to the halm axis and the degree of development of the mechanical tissue of leaves." Somewhat similar observations were made by the authors on plants which had been inoculated with an aqueous conidial suspension. However, a more uniform distribution of leaf lesions was obtained by suspending the conidia in a sodium oleate–gelatin solution at the time of inoculation.

Abe (4) also noted, in the case of grown plants, that the disease outbreak occurred at the greatest frequency on the spikelets, but that this type of infection resulted in only a negligible reduction in yield; whereas infection "on all parts of the pedicels of spikes and the second internode of halms tended to show the greatest influence on the yield of rice kernels. . . ." In general, these observations agree with those made by the authors except that under controlled conditions severe infection of the spikelets resulted in a high reduction in yield, especially when the heads were inoculated during the early flowering stage.

Suzuki (19) concluded from his studies on the anatomical differences between susceptible and resistant plants that "the thickness of the outer walls and of the silicated outermost layer as well as the number of the silicated bulliform cells, of the silicated long or short cells, and of the silicated stomata seems to be closely correlated with the susceptibility of the rice plant to blast disease, while the number of stomata is not considered to be in close correlation with the susceptibility of the plant to the disease." It was not determined whether the increased resistance noted in the leaves and sheaths of plants in the later stages of development, as observed in our work, was of the same nature as that observed by Suzuki (19).

The plants which were inoculated in the later stages of development were grown to maturity and the panicles harvested to determine the percentage of blasting that had occurred (Table 2). The Acadia variety received only one inoculation while the Onsen variety was inoculated a second time approximately two weeks later to simulate secondary spread. It is evident from these results that *Piricularia oryzae* is capable of causing extensive damage to a rice crop which is in its later stages of development.

ENVIRONMENTAL FACTORS RELATED TO INFECTION

Period of Continued Wetness of the Plants

This environmental factor is a result of an interaction of several other factors such as relative humidity, air temperature, dew point, etc. It has

been considered to be one of the most important factors governing the establishment of primary infection and secondary spread in a rice field.   Hemmi and Abe (9) found in their experiments that continuous exposure to wetness for 6 to 8 hours at temperatures between 20° and 28° C. was necessary for the infection of rice seedlings.

In order to determine the period of continued wetness necessary for the establishment of initial infection on rice at temperatures optimum for the growth of the fungus (24–28° C.), a series of potted seedling rice plants in the 3–5-leaf stage was inoculated with a suspension of conidia of *Piricularia oryzae* and placed in the humidity tent.   Duplicate pots were removed at periodic intervals from 4 to 24 hours after inoculation.   The number of lesions per infected leaf was determined after 5 days and the number of dead and infected leaves after 14 days (Table 3).   These results were

TABLE 3.—*The influence of length of exposure to continued wetness on the amount of infection by Piricularia oryzae*[a]

Time in tent	Dead leaves per plant	Infected leaves per plant	Leaf spots per infected leaf
Hours	*Number*	*Number*	*Number*
4	0.0	0.0	0.0
8	0.0	0.0	0.0
10	0.0	0.5	1–3
12	0.0	1.2	1–10
14	0.1	1.3	1–10
16	0.1	2.1	1–10
18	0.3	1.7	1–10
20	0.2	1.5	1–10
24	0.2	0.9	1–10
(control)	0.0	0.0	0.0

[a] Data represent averages from 30 plants in each treatment.

similar to those found in previous experiments at slightly lower temperatures (20–22° C.), when 10 hours was the shortest time of exposure that resulted in infection.   At these lower temperatures a period of 20 to 24 hours was necessary for the establishment of maximum infection.   It was concluded that 16 to 24 hours of high humidity and continued wetness of the plants at 24–28° C. would cause maximum infection.

When it was discovered that a 16- to 24-hour period was necessary for the establishment of maximum infection on plants if the spores were applied directly, it was presumed that a longer period would be necessary for natural secondary spread to take place.   The period of continued wetness necessary for secondary spread and the establishment of infection on rice at 24–28° C. was determined by exposing noninfected rice plants together with infected plants in the humidity tent.   In one of these experiments, 4 pots of noninfected Onsen rice were placed in the center of the humidity tent and several infected plants were placed at each end with 2–3 feet between the healthy and infected plants.   The 4 pots of healthy plants and the infected plants were exposed for 24 hours to continued wetness and to the air currents pro-

duced by the humidifier. At the end of the 24 hours the 4 pots were removed and replaced by 4 other pots of healthy rice. The water was turned off and the plants were exposed only to the air currents from the humidifier. After a 24-hour exposure all the original infected plants were removed and the 4 remaining pots of rice were exposed to 24 hours of continued wetness. The first series, which was exposed to continued wetness and secondary spread for 24 hours, had an average of 2.8 infected leaves per plant with leaf lesions too numerous to count. The second series, which was exposed under dry conditions of secondary spread, had an average of 0.3 infected leaves per plant, and there were very few lesions on these leaves. Thus it seemed that the conidia spread from the diseased to the healthy plants more readily during the wet period than during the dry period.

TABLE 4.—*The influence of time of exposure to continued wetness on the spread of Piricularia oryzae from infected to noninfected rice plants*

Time exposed	Additional exposure to high humidity	Number of plants	Leaf spots per plant	Infected leaves per plant
Hours	*Hours*		*Av.*	*Av.*
3	0	36	0	0.0
5	0	34	0	0.0
7	0	29	0	0.0
9	0	34	0	0.0
11	0	32	0	0.0
13	0	38	Trace	Trace
15	0	34	1–10	0.3
21½	0	32	50+	2.6
3	18	33	Trace	Trace
5	18	36	Trace	Trace
7	18	36	1–10	0.1
9	18	38	0	0.0
11	18	37	1–10	0.2
13	18	34	1–10	0.3
15	18	33	10–20	0.5
21½	18	32	50+	2.2

The same infected plants that served as a source of inoculum in the previous experiment were placed at both ends of the humidity tent and 32 pots of healthy Onsen rice seedlings were placed in the center of the tent. Sets of 4 pots were removed from the center of the tent after exposure for the time intervals given in column 1 of table 4. The plants that served as a source of inoculum were discarded at the end of 21½ hours. Twenty-four hours after the last pots were removed, 2 from each set of 4 were replaced and subjected to 18 additional hours of continued wetness (Table 4). Very little secondary spread occurred during the first 15 hours of exposure to continued wetness even when the exposed plants were resubjected to conditions favorable for infection. Since the most infection was established in 21½ hours, it would indicate that an exposure to continued wetness for an equal or greater length of time would be necessary in the field for maximum secondary spread and probable establishment or initiation of an epiphytotic.

The results obtained in the greenhouse were confirmed by an experiment in Texas on initial infection and subsequent secondary spread. *Piricularia oryzae* was not present naturally in the proximity of the tests. A 12 × 12 foot plot was selected in a field of Onsen rice and was inoculated with a conidial suspension of *P. oryzae* using the same rate of application as in the greenhouse experiments. The plants were in the seedling–early tillering stage. Immediately after inoculation the plants were exposed to a 14-hour period of continued wetness by dew and water of guttation which was sufficient to establish heavy infection. Lesions resulting from secondary spread were evident on rice plants at distances of 100 to 200 feet from the original inoculated plot within 3 weeks. The infection resulting from secondary spread was limited to very few leaf spots, and even in the plot itself infection was more or less comparable to the amount of spread that took place in a 10–15-hour period in the controlled humidity tent in the greenhouse. Proof of the spread was established by taking samples of infected leaves and placing them in a moist chamber to sporulate. After the rice ripened, 4 one sq. yd. areas were harvested from the plot and 4 similar areas were selected about 25 feet from the plot and harvested for controls. An average of 179 grams of rice was recovered from each sample area in the treated plot as compared to 260 grams from the control areas. Although there was a 100 per cent variation between replicate samples, it appeared that the pathogen was at least partially responsible for the reduction in yield. Thus it appeared that one reason for the absence of *P. oryzae* in the Beaumont, Texas, rice area was the lack of conditions favorable to secondary spread.

Periodic Wetting

No infection by *Piricularia oryzae* occurred when the inoculated plants were kept in the humidity tent for 8 hours or less (Table 3). Periods of continued wetness in nature are often less than 8 hours. It then seemed desirable to learn the effect on the amount of infection a relatively short period of wetting would have when it was followed later by a period of wetting sufficient in itself to allow the establishment of infection. Even though no infection occurred with 8 hours' exposure to continued wetness, it was known that the conidia would germinate on the leaf of the host within this time.

In order to determine the effect of short periods of wetting upon the establishment of infection, 14 pots of rice containing 15 plants per pot were each inoculated with a suspension of conidia of *Piricularia oryzae*. Duplicate pots were used for each treatment. The plants were inoculated with the same spore suspension at different times so that after exposure to continued wetness for desired lengths of time, they were all removed from the tent and placed on a greenhouse bench in the sun at 8 A.M. Ten hours later all the plants were replaced in the humidity tent for an additional 18 hours (Table 5). Plants exposed to wetting for 8 hours or less (followed by a dry and a second wet period) had 30 to 50 per cent of the amount of

infection obtained with the 10-hr. period. The greater amount of infection with the 10 and 12 hours of initial wetting was probably due to (1) infection already established when the drying began, and (2) the killing of some germinated conidia by drying on those plants exposed for shorter periods.

Period of Reinfection

From the standpoint of secondary spread in the field, it was of interest to determine the length of time elapsing between the establishment of initial or primary infection and the development of lesions on which sporulation would occur, thus providing a source of secondary inoculum. In addition, an attempt was made to determine the period of greatest conidial production and subsequent secondary spread from the new lesions. This was accomplished by inoculating a series of 40 pots of rice plants in the seedling

TABLE 5.-- *The effect of periodic wetting of the plants on infection by Piricularia oryzae*

Initial period of wetting[a]	Av. no. dead leaves per plant	Av. no. infected leaves per plant	No. lesions per infected leaf
Hours			
2	0.1	0.9	1–5
4	0.0	0.7	1–5
6	0.1	0.9	1–5
8	0.0	0.7	1–5
10	0.7	1.8	1–10
12	0.4	1.3	1–10

[a] All plants had 10 hours of dry atmosphere, then 18 hours of wetting after the initial period of wetting. See table 3 for results obtained when plants received only the initial period of wetting.

stage and placing them in the humidity tent for 24 hours to establish primary infection. Duplicate pots from this series were replaced in the humidity tent at regular intervals after the initial inoculation to determine to what extent the infected plants would serve as sources of inoculum for their reinfections. The lesions on all those plants replaced between 6 and 10 days after the initial inoculation produced abundant conidia which resulted in the establishment of much secondary infection. No secondary infection occurred on plants replaced after 2 and 4 days. After 10 days, little secondary infection occurred and the plants were able to outgrow the initial infection. Thus it appeared that a minimum period of about 6 days after infection was necessary for the lesions to develop to the stage at which they were capable of producing conidia which in turn acted as inoculum for secondary spread.

Longevity of Conidia

One of the factors which determines the amount of infection that can be expected is the ability of the conidia to survive on plants in the field. The importance of this factor was determined by inoculating a series of 76 pots of Onsen rice and placing them on the greenhouse bench. One half of the

series was inoculated with a conidial suspension and the other half with a dry conidial dust. Four pots of rice from each series were placed in the humidity tent for a 24-hour period at daily intervals for the first four days and at 2-day intervals for the next 8 days. *Piricularia oryzae* developed only on those plants placed in the humidity tent from 1 to 6 days after inoculation. Thus conidia were able to remain viable on dry plants for 6 days under greenhouse conditions.

Sueda (15) suggested that conidia would survive fairly long when floating on the surface of the water in a rice field, but that when submerged they would die within two weeks. This introduced the question of the importance of irrigation water on the spread of *Piricularia oryzae* in the field. It appeared difficult actually to study the survival of the conidia in the field, so an experiment was conducted in the laboratory to determine the extent of survival of conidia in suspension at various temperatures (Table 6). In

TABLE 6.—*The viability of Piricularia oryzae conidia after storage at four temperatures for varying lengths of time in water suspension*

Time in days	Temperature in degrees C.			
	8	20	28–30	32
	Percentage germination[a]			
0	.	.	99	.
0.25	100	99	100	99
1	97	91	38	9
2	98	52	5	0
3	100	22	1	1
5	100	5	1	1
8	100	0	0	0
14	80	0	0	0

[a] Based upon 100–250 conidia for each sample.

this experiment 50-ml. samples of conidial suspension containing 90,000 conidia per ml. were taken from a 7-day-old oats-sorghum culture and placed in 22-mm. test tubes. One tube was stored at each of the following temperatures: 8° C., 20° C., room (28–30° C.) and 32° C. At periodic intervals the tubes were shaken and samples taken for germination tests.

The viability of the conidia decreased rapidly during the first 24 hours in water suspension at 28–32° C. At 20° C. the rapid decline in viability was between 24 and 48 hours. After 8 days' storage at 8° C., the viability was 100 per cent but dropped to 80 per cent after 14 days in storage. Irrigation water temperatures in Texas were recorded for one week, in the early morning, at noon, and in the evening. The lowest temperature recorded was 31° C. and the highest 38° C. During this same period the daily minimum air temperature was 22° C. and the maximum 35° C. Thus it appears that few conidia would be able to survive in irrigation water more than 24 hours.

While the work with *Piricularia oryzae* was in progress, it was constantly observed that conidia did not germinate in suspensions unless they were

floating on the surface or were suspended in a shallow layer of water on a glass slide. This would indicate the probable lack of sufficient oxygen for germination. It was also observed that the conidia floating on the surface of water nearly always germinated if the temperature of the water was above 20° C. Taking these results and observations into consideration, it seems highly improbable that the irrigation water would serve as a common method for the dissemination and spread of conidia of *P. oryzae*.

Inhibition by Helminthosporium oryzae

Aoki (7) reported the suppression of germination of *Piricularia oryzae* conidia in contact with conidia of *Helminthosporium oryzae*. Hemmi *et al.* (10) stated that the presence of *H. oryzae* conidia resulted in a reduction of both germination and length of germ tube of *P. oryzae* when the two pathogens were applied simultaneously to rice plants. The present investi-

TABLE 7.—*The apparently inhibitory effect of Helminthosporium oryzae on Piricularia oryzae infection*

Inoculum	Replicate	P. oryzae lesions[a]	
		Total	Average
P. oryzae conidia	1	337	
	2	332	370
	3	442	
P. oryzae conidia plus H. oryzae mycelium	1	7	
	2	59	42
	3	49	
P. oryzae conidia plus H. oryzae conidia ..	1	5	
	2	15	14
	3	21	

[a] Each number represents lesions on 15 plants.

gation showed no evident antagonism between the two organisms when grown together on rice-polish agar or when the conidia of each were allowed to germinate together in water. In several experiments using the two pathogens together on plants, no definite conclusions could be drawn because of the erratic host response to inoculations with *P. oryzae*. In one experiment, however, it was shown that *H. oryzae* did reduce the development of *P. oryzae*. In this experiment a series of Onsen plants was inoculated in the greenhouse with ground up dried mycelium from *H. oryzae*, dried *H. oryzae* conidia, *P. oryzae* conidia, and with mixtures of the latter with the two forms of *H. oryzae* inoculum. In all cases *H. oryzae* infection was heavy. The number of *P. oryzae* lesions on plants inoculated with the two agents was markedly reduced below that where *P. oryzae* was used alone (Table 7). Although experiments showed no evident antagonism between the two pathogens when grown together on rice-polish agar or when the conidia of both were allowed to germinate together in water, the development of *P. oryzae* was reduced by the presence of *H. oryzae* when plants were inoculated simultaneously with the two fungi.

Other Cereal Hosts

Anstead (6) presented experimental evidence of the ability of *Piricularia oryzae* from rice to attack wheat.   Sundararaman (16) reported successful cross inoculation of *P. oryzae* from rice to oats, ragi (*Eleusine coracana*), and wheat, and vice versa.   In the following experiments, wheat, oats, barley, corn, rye, and sorghum seedlings were inoculated with a conidial suspension of *P. oryzae*, and placed in the humidity tent for 24 hours.   Lesions appeared on barley, corn, rye, and wheat from 5 to 7 days after inoculation. Sorghum and oats had no lesions.   Infected leaves of barley, corn, rye, and wheat were placed in glass moist chambers and examined 24 hours later. Conidia were produced in abundance on all the lesions examined on all four species.   It was not ascertained whether these cereals were susceptible to *P. oryzae* in the field.

DISCUSSION

Because of the importance of the "blast" of rice in Asiatic countries, many of the factors influencing infection have been investigated and reported upon in the literature.   Several significant points were demonstrated by the investigations reported in this paper.

The question of relative susceptibility of plant parts at different growth stages indicates that the inoculations should be made during the seedling stage because even susceptible varieties become resistant in the late tillering and early heading stages.   Information in regard to susceptibility to panicle infection would have to be obtained from inoculations in the heading stage or as a result of secondary infection initiated from lesions established during the early stages of development.

Infectivity was also considered from the standpoint of the pathogen.   In this case the moisture relationships were of major importance, especially the effect of continued wetness of the plants.   It was found that 16 to 24 hours of continued wetness were necessary for the establishment of maximum primary infection at temperatures between 22° and 28° C., and in no case was infection established on plants in less than 10 hours.   If the plants were subjected to periodic wetness, infectivity was held to 30–50 per cent of the maximum when the initial exposure period to continued wetness was 8 hours or less.

The period of continued wetness of the plants also was an important factor with respect to secondary spread.   Experimental evidence obtained in greenhouse studies indicated that only a few conidia were liberated while the plants were dry.   On the other hand, when they were kept wet the amount of secondary spread was extensive after 24 hours of exposure.   Thus it appears that plants had to be wet before conidia would be liberated to infect other healthy leaves and plants.   In addition, it was shown that only a slight amount of secondary spread would take place within a 15-hour period from infected plants which already had conidia on primary lesions, and that approximately 21½ hours of continued wetness were necessary for heavy infection.

These results may possibly explain the difficulties encountered while attempting to study the infectivity and spread of *Piricularia oryzae* at Beaumont, Texas, during the summers of 1944–45. Primary infection was readily established in the field by artificial inoculation of Onsen rice in its early stages of development. This was because of the nightly occurrence of heavy dews, which generally lasted from 12 to 14 hours, and favorable seasonal temperatures. It was expected that a correspondingly heavy amount of secondary spread would take place after heavy primary infection was established. This was not the case. The actual number of leaf lesions was few. Even when the plants reached the heading stage there was only a slight amount of culm and head infection. Knowing the factors necessary for optimum secondary spread, it appeared that the one favorable environmental condition lacking in Texas was the presence of continuously wet weather for periods greater than 24 hours. The daily weather conditions in Texas were such that as much as 24 hours of continued wetness was a rare occurrence in 1945 (with the possible exception of hurricanes). Thus, plants were never exposed to more than 12–14 hours of free moisture at a time. There was considerable rain, but only in showers followed by sunshine so that the plants dried immediately afterward. Apparently the conditions in the Beaumont area are not favorable for the secondary spread of *P. oryzae*.

One other consideration of importance was the presence of *Helminthosporium oryzae* infection in the rice in Texas. In greenhouse experiments, where the two pathogens were used together to inoculate plants, *H. oryzae* reduced the development of *Piricularia oryzae*. During the early part of the season of 1945, *H. oryzae* infection was light and was not considered of importance in reducing the spread and infectivity of *P. oryzae*. It may have been of considerable importance during the latter part of the season when the plants were in the flowering stage. At this time, the rice was thoroughly infested with *H. oryzae* and difficulty was encountered in establishing infection by *P. oryzae* even in the heading stage.

There appears to be no reason to believe that *Piricularia oryzae* conidia will survive for any great length of time in the irrigation water of the field. In laboratory studies, conidia stored in water at 32° C. lost their viability in 1 day; at 28–30°, in 2 days; and at 20° C., in 3–4 days. Those conidia that float on the surface of the water germinate within a few hours. Therefore, it seems highly improbable that the spores would survive for any length of time in irrigation water. In rice-growing areas there is nearly always heavy dew formation every night. This would mean that spores would be exposed to periodic wetness, and that too would tend to reduce the viability of the conidia.

SUMMARY

1. Rice plants were most susceptible to *Piricularia oryzae* in the seedling, early tillering, and heading stages.

2. Exposure of inoculated plants to continued wetness for 16 to 24 hours

at temperatures between 24° and 28° C. resulted in maximum *Piricularia oryzae* infection.   No infection resulted from exposure of less than 10 hours.

3. Inoculated plants exposed to a wetting period of 8 hours or less followed by a dry and a second wet period, had from 30 to 50 per cent of the maximum amount of infection.

4. High humidity and the presence of free moisture on the leaves of infected plants were essential for secondary spread and the establishment of infection of rice plants.   Under such conditions a 15-hour exposure was not sufficient to allow secondary infection to any significant degree, but a 21½-hour period resulted in secondary spread and the establishment of a high degree of infection.

5. A minimum period of 6 days after inoculation was necessary for lesions to develop to a stage capable of producing conidia which, in turn, acted as inoculum for secondary spread.

6. Conidia remained viable on dry plants for 6 days in the greenhouse. In suspension, the viability decreased rapidly the first 24 hours under storage at temperatures between 28° and 32° C.   Since these were below the normal temperatures of irrigation water in Texas rice fields, it is believed that conidia would not be able to survive in irrigation water for more than 24 hours and thus it seems improbable that the irrigation water would serve as a common method for the dissemination of conidia of *Piricularia oryzae*.

7. No antagonism was noted between conidia of *Piricularia oryzae* and *Helminthosporium oryzae* when they were grown together on rice-polish agar or when the conidia of each were allowed to germinate together in water.   When the two were combined and used for plant inoculations, *H. oryzae* reduced the amount of disease produced by *P. oryzae*.

8. Eighteen short-grain and 2 medium-grain rice varieties were tested in the seedling (3–5-leaf) stage for their relative susceptibility to *Piricularia oryzae*.   Of these, 11 were classified as being very susceptible or susceptible, one as resistant, and the other intermediate in reaction.

9. Barley, corn, rye, and wheat in the seedling stage of development were found to be susceptible to infection by *Piricularia oryzae*.

CAMP DETRICK,
   FREDERICK, MARYLAND.

LITERATURE CITED

1. ABE, T.   On the effect of sunlight on the infection of the rice plant by *Piricularia oryzae*.   Forsch. auf dem Geb. der Pflanzenkrankh. (Kyoto) 1: 46–53.   1931. (In Japanese, Abstr. in Rev. Appl. Mycol. 11: 800.   1932.)

2. ————————.   On the influence of soil temperature upon the development of the blast disease of rice.   Forsch. auf dem Geb. der Pflanzenkrankh. (Kyoto) 2: 30–54.   1933.   (Japanese with English summary.)

3. ————————.   On the relation of atmospheric humidity to the infection of the rice plant by *Piricularia oryzae* B. et C.   Forsch. auf dem Geb. der Pflanzenkrankh. (Kyoto) 2: 98–124.   1933.   (Japanese with English summary.)

4. ————————.   On the relation of susceptibility of different portions of the rice-plant to the blast fungus, *Piricularia oryzae* Br. et Cav.   Forsch. auf dem Geb. der Pflanzenkrankh. (Kyoto) 3: 115–136.   1937.   (Japanese with English summary.)

5. ANDERSEN, A. L., and B. W. HENRY. The use of wetting and adhesive agents to increase the effectiveness of conidial suspensions for plant inoculations. Phytopath. **36**: 1058–1059. 1946.

6. ANSTEAD, R. D. Report on the operations of the Department of Agriculture, Madras Presidency, for the official year 1922–23. 30 pp. 1924.

7. AOKI, K. Physiological studies on the conidial germination of *Piricularia oryzae* and *Ophiobolus miyabeanus*. Forsch. auf dem Geb. der Pflanzenkrankh. (Kyoto) **3**: 147–176. 1937. (Japanese with English summary.)

8. HEMMI, T. On the relation of soil moisture to the development of the rice blast disease. Agr. and Hort. **4**: 1143–1154. 1929. (In Japanese. English abstract in Jap. Jour. Bot. **5**: (4). 1930.)

9. ——————, and T. ABE. On the relation of temperature and period of continuous wetting to the infection of the rice plant by *Piricularia oryzae*. Forsch. auf dem Geb. der Pflanzenkrankh. (Kyoto) **1**: 33–45. 1931. (Japanese with English summary.)

10. ——————, D. IKEYA, and Y. INOUE. Influence of *Ophiobolus miyabeanus* on the penetration of *Piricularia oryzae*, in the host body. Agr. and Hort. **11**: 953–964. 1936. (In Japanese. English abstract in Jap. Jour. Bot. **8**: (99). 1937.)

11. ——————, and J. IMURA. On the relation of air-humidity to conidial formation in the rice blast fungus, *Piricularia oryzae*, and the characteristics in the germinations of conidia produced by strains showing different pathogenicity. Ann. Phytopath. Soc. Japan **9**: 147–156. 1939. (Japanese with English summary.)

12. IMURA, J. On the effect of sunlight upon the enlargement of lesions of the rice blast disease. Ann. Phytopath. Soc. Japan **8**: 23–33. 1938. (Japanese with English summary.)

13. SAKAMOTO, M. On the facilitated infection of the rice blast fungus, *Piricularia oryzae* Cav. due to the wind. Ann. Phytopath. Soc. Japan **10**: 119–126. 1940. (Japanese with English summary.)

14. SAWADA, K. Rice blast disease and manuring 2–9. Agr. Rept. Formosa **35**: 22–31, 111–118, 182–189, 251–259, 383–387, 444–449, 655–656. 1937. (In Japanese. English abstract in Jap. Jour. Bot. **10**: (62)–(63). 1940.)

15. SUEDA, H. Studies on the rice blast disease. Rept. Dept. Agric., Govt. Res. Inst. Formosa, No. 36. 1928. (In Japanese. English abstract in Jap. Jour. Bot. **4**: (73). 1929.)

16. SUNDARARAMAN, S. Board of Agr. Proc. 3rd meeting of Mycological workers in India, Feb., 1921.

17. SUZUKI, H. On the relation of soil moisture to the development of the blast disease of rice plant, with special reference to the results of inoculation experiments on seedlings and pedicels of spikes of plants grown on soils differing in the time and duration of drying and irrigation. Forsch. auf dem Geb. der Pflanzenkrankh. (Kyoto) **2**: 172–185. 1933. (Japanese with English summary.)

18. ——————. Studies on the influence of some environmental factors on the susceptibility of the rice plant to blast and Helminthosporium diseases on the anatomical characters of the plant. III. Influence of differences in soil moisture and in the amount of nitrogenous fertilizer given. Jour. Coll. Agr. Tokyo **13**: 277–331. 1934.

19. ——————. Studies on the relations between the anatomical characters of the rice plant and its susceptibility to blast disease. Jour. Coll. Agr. Tokyo **14**: 181–264. 1937.

20. THOMAS, K. M. Detailed administration report of the Government Mycologist, Madras, for the year 1937–38. 1938. (Abstr. in Rev. Appl. Mycol. **18**: 88–89. 1939.)

21. TOCHINAI, Y., and S. KOMUJA. Studies on the infection of *Piricularia oryzae* Br. and Cav. on maltreated rice plants. Jour. Fac. Agr. Hokkaido Univ. **44**: 33–76. 1939. (Abstr. in Rev. Appl. Mycol. **18**: 546. 1939.)

SOYBEAN DISEASES IN ONTARIO AND EFFECTIVENESS
OF SEED TREATMENT[1]

A . A . H I L D E B R A N D   A N D   L . W . K O C H[2]

Co-incident with a rapidly increasing acreage of soybeans in southwestern Ontario during recent years has been the discovery of a corresponding increase in the number and diversity of the diseases that affect this crop. To what extent seed treatment might prove efficacious in controlling certain of the seed-borne diseases of the district seemed to the writers to be one of the problems warranting immediate investigation. Consequently, studies in this connection and along related lines were undertaken in 1943, and the results obtained during the past three seasons are reported in the present paper.

Soybeans were first grown in Canada about 50 years ago (6, 17). For many years they attracted comparatively little attention as is shown by the fact that until 1940 the average acreage in the Dominion was only 10,000 acres (6). In 1942, however, the acreage increased more than fourfold to 41,490 acres yielding a total of 912,000 bushels (19). In 1944, the soybean crop in Canada totalled approximately 45,000 acres, of which 44,700 yielding 845,000 bushels valued at $1,690,000 were grown in the province of Ontario (6, 20). Thus, to date, commercial production of soybeans has been confined almost entirely to Ontario, where conditions are most favorable for their growth and where prospects for marketing them on a basis of large-scale production have greatly improved (2). In Ontario, production of soybeans is not uniformly province-wide, for of the 1944 total of 44,700 acres, 35,400 acres were concentrated in the counties of Essex, Kent, Middlesex, Norfolk, and Lambton in southwestern Ontario.

In 1926, Drayton (7) reported the occurrence of three diseases of the soybean in Canada. The number of parasitic diseases now known to have been found in soybean-growing areas variously scattered across the Dominion from Nova Scotia to British Columbia, has reached a total of 13,[3] which figure represents approximately half of the known number of parasitic diseases of the soybean. Because of the fact that large-scale production of soybeans is more concentrated in southwestern Ontario than elsewhere in Canada, it is of especial interest and significance that (a) all 13 diseases of the soybean known to occur in Canada have been found within this relatively restricted area (Table 1), (b) 8 of them have not yet been reported

as occurring elsewhere in the Dominion, (c) 10 of them have been found within the area since 1942 (4), and (d) at least 8—possibly 9—of them are known to be seed-borne (Table 1).

TABLE 1.—*Parasitic diseases of soybeans reported as occurring in southwestern Ontario*

Common name	Causal organism	Date first reported	Seed-borne	Not yet reported elsewhere in Canada
1. Bacterial leaf spot or bacterial blight	*Pseudomonas glycines* (Coerper) Stapp	1924	+	
2. Soybean mosaic	Soja virus 1	1924	+	
3. Downy mildew	*Peronospora manchurica* (Naoumoff) Sydow	1935	+	
4. Anthracnose	*Colletotrichum glycines* (Hori)	1942	+	
5. Phyllosticta leaf spot	*Phyllosticta sojaecola* Massl.	1943	+	
6. Pod and stem blight	*Diaporthe phaseolorum* (Cke. and Ell.) Sacc. var. *sojae* (Lehm.) Wehmeyer	1942	+	+
7. Fusarium blight	*Fusarium oxysporum* Schl. f. *tracheiphilum* Snyder and Hansen	1942		+
8. Frog-eye	*Cercospora sojina* Hara	1943	+	+
9. Brown spot	*Septoria glycines* Hemmi	1943	+	+
10. Bud blight, top necrosis, streak	Virus of tobacco-ring-spot group	1944		+
11. Charcoal rot	*Macrophomina phaseoli* (Maubl.) Ashby	1944		+
12. Stem rot	*Sclerotinia sclerotiorum* (Lib.) de Bary	1942		+
13. Lavender spot or purple spot	(Presumably) *Cercosporina kikuchii* Mats. and Tomo.	1942	?	+

LITERATURE REVIEW

Pioneering in North America in seed treatment of soybeans, Wolf and Lehman (28) in 1926 and Lehman (16) in 1929 reported that certain of a large number of fungicides under test increased the stand of plants and gave good control of downy mildew, whereas others reduced the stand and failed to control such diseases as frog-eye leaf spot and bacterial pustule.

In greenhouse trials in Oklahoma in 1941 and 1942, Davy (5) found that treatment of Virginia soybean seed with New Improved Ceresan and Spergon effectively prevented seed rots and pre-emergence damping-off in soil naturally infested with *Rhizoctonia solani* Kühn.

In a field test in Minnesota in 1942, Tervet (25) obtained no clear evidence that seed treatment with New Improved Semesan Jr. and Spergon controlled any of the seed-borne diseases.

In 1943, Koehler (13) called attention to coordinated interstate greenhouse tests, preliminary results of which indicated that when germinability of soybean seed is low as a result of too high a moisture content, emergence can be increased by treatment with Ceresan, Spergon, or Arasan.

According to Johnson and Koehler (11) in 1943, greenhouse tests were carried out in Illinois, using Semesan Jr., Cuprocide, Barbak C, and Spergon. Cuprocide failed to benefit emergence but the other protectants caused a significant average increase in stand.

In Iowa in 1943, Melhus *et al.* (18) investigated the effect of seed treatments on soybeans, the results of which indicated that though the stand was increased by certain materials, the yield remained unaffected.

In 1943, Petty (21) reported that the use of Arasan, Spergon, and New Improved Ceresan in Maryland tests resulted in about 10 per cent increase in stand.

Heuberger and Manns (9, 10), reporting, in 1943, on tests carried out in Delaware, stated that Arasan, Spergon, Ceresan, and Dow No. 5 accelerated emergence of seedlings and increased final stand of plants; Arasan, however, being the only material to increase the yield significantly.

In summarizing his investigations on the influence of fungi on storage, seed viability, and seedling vigor of soybeans, Tervet (26, 27) stated that severe retardation in seedling growth resulted from storage conditions favoring the developing of *Aspergillus spp.*, but seed treatment with the maximum adhesive load of Arasan improved the vigor and stand of plants.

From Puerto Rico, Stoddard (24) reported, in 1944, that treatment of Seminole soybean seed with Arasan, Semesan, and Spergon gave valuable protection against seed rots and pre-emergence damping-off.

Porter (22) and Koehler (14), in 1944, and Allington *et al.* (1), in 1945, reported the results of coordinated, interstate seed-treatment projects involving tests on oil-type and edible soybeans with Spergon, Arasan, Fermate, Semesan Jr., and New Improved Ceresan. While in many instances significant increases in stand were obtained from treatment, nevertheless, in general, yield remained unaffected. Koehler (14) expressed the opinion that tests must be continued over a period of years before definite conclusions can be reached; the same author (15) and Allington and co-workers (1) are doubtful if treatments will be beneficial, or should be recommended, except in a year following a season in which the seed has undergone severe weather damage.

The work of Kiesselbach (12) and of Bartholomew (3) offers no suggestion that significant benefits are to be derived from the treatment of soybean seed with plant hormones.

MATERIALS AND METHODS

Seed of the variety A. K. Harrow, which is grown most extensively in Essex county and which, therefore, was used in the present investigations, was supplied by the Harrow Experimental Station or obtained as required from various commercial sources in the district. Examination of seed and, where necessary, segregation into different categories was done by hand-sorting with the aid of a dissecting microscope. The protectants used were Spergon (tetrachloro-parabenzoquinone), Arasan (tetramethyl thiuramdi-

sulphide), and Fermate (ferric dimethyldithiocarbamate), the first-mentioned at the rate of 3 oz. (0.312 per cent), the two latter at 2 oz. (0.208 per cent) per bu. Treatment was during the first week in February each year, following which the seed was packaged and stored at laboratory temperatures until planting time in May. Each year, prior to spring ploughing, the soil, a Fox sandy loam, received an application of a 2–12–10 commercial fertilizer applied broadcast at the rate of 250 lb. per acre. The addition of a legume inoculant was not considered necessary since crops of soybeans had been recently grown on the site selected for the experiment. All planting was done by hand in a randomized, 5-replicate design, the test unit within each plot comprising duplicate rows, 35 ft. long in 1944, 30 ft. in 1945. The rows were spaced 30 inches apart and, in the seed-treatment experiments, were planted at the rates of 35 and 30 lb. per acre, in 1944 and 1945, respectively.

Periodic examinations, begun a few days after planting and continued throughout the season, furnished the data on emergence and the incidence

TABLE 2.—*Comparison of 1942 poor seed and 1943 good seed*

Year	Percentage			
	Healthy seed	Diseased seed	Broken seed and trash	Germinability
1942	54.29	39.84 [a]	5.85	23.4
1943	85.73	2.59	11.68	87.2

[a] This diseased portion comprised the following sub-categories: mildewed or moldy seed, 77.11 per cent; dwarfed and distorted seed, 6.21 per cent; discolored seed coat, 12.42 per cent; wrinkled seed coat, 4.23 per cent.

and severity of disease. In harvesting, the plants were cut near the base of the stem with pruning shears, assembled in bundles, and threshed[4] the same day. All weighings and necessary moisture determinations were made immediately after threshing.

TREATMENT OF DIFFERENT SEED LOTS

Due to extremely unfavorable weather conditions in 1942, soybean seed produced in southwestern Ontario that year was, in general, the poorest for many years, not only being low in viability but also high in disease potentiality.[5] In marked contrast, seed produced in 1943 was of outstandingly high quality. Eight-pound, composite samples of 1942 and 1943 seed, being offered for sale in the district for seeding purposes, were compared as to their germinability and also segregated by hand-sorting into their various component categories with results as shown in table 2.

With seed-lots of two successive years showing such marked differences, the opportunity to test the effect of seed treatment seemed to be an especially

[4] J. L. Jones, No. 4, pea and bean thresher.
[5] This term refers to the capacity of the seed to induce disease in the progeny to which it gives rise.

excellent one.   Consequently, on May 15, 1944, treated as well as nontreated seed of the variety A. K. Harrow produced in 1942 were planted at the rate and in accordance with the methods described.   Two days later, May 17, the experiment was repeated in exact replica except that the higher quality 1943 seed was used.   On May 24, nine days after planting, the seedlings from 1942 seed were breaking ground.   On May 29, fourteen days from date of planting, the Spergon-treated rows in all 5 replications were superior to all others not only because of more uniform emergence but also because of outstanding vigor of seedlings.   This superiority was readily noticeable until well on into the growing season.   In general, seedlings from 1943 seed broke ground earlier and much more uniformly than did those from 1942 seed.   On May 29, twelve days after planting, it was apparent that in all 5 replications for 1943 seed Spergon-treated rows were superior to the others, but this superiority was not so marked as in replications for 1942 seed.

TABLE 3.—*Emergence, incidence of disease and abnormality, and yield in relation to difference in quality and treatment of A. K. Harrow 1942 and 1943 soybean seed*

Treatment	Total emergence		Diseased and abnormal seedlings in per cent		Yield in pounds			
					1942		1943	
	1942	1943	1942	1943	Total	Av.	Total	Av.
Fermate	256	1148	21.8	3.9	23.9	5.9	26.3	6.5
Arasan	283	1145	21.2	6.5	22.9	5.7	25.9	6.4
Spergon	712	1280	14.3	1.3	30.9	7.7 [a]	25.8	6.4
Check	348	1154	22.4	6.4	24.9	6.2	26.1	6.5
Total	1599	4727			102.6		104.1	.....

[a] Highly significant over Arasan, Fermate, and Check. L.S.D. for averages, 1 per cent = 1.47; 5 per cent = 1.02.

Three weeks after planting, emergence of seedlings and incidence of diseased and abnormal seedlings[6] were recorded, and on October 11 the plots were harvested and yields determined.   Data for the two experiments are summarized in table 3.

The germinability of the 1942 seed was extremely low as compared with that of the seed produced in 1943.   The nontreated 1942 seed, for example, produced only 348 seedlings, whereas from the 1943 nontreated seed planted at the same rate, 1154 seedlings developed.   With both the poor and the good seed, Spergon treatment gave highest emergence of seedlings.   Fermate and Arasan, in marked contrast to Spergon, apparently depressed emergence of the 1942 poor seed even below that of the nontreated check.   This seemingly deleterious effect was not observed, however, in the case of the 1943 good seed.

[6] Among diseased and abnormal seedlings were included those which, by discoloration or necrosis of hypocotyl, cotyledon, or first-formed pair of true leaves, showed positive evidence of a diseased condition, or which, by distortion or malformation of the above-mentioned parts or by delayed development following slower emergence, differentiated themselves readily from the normally developing seedlings of corresponding age.

The incidence of disease and abnormality was relatively much higher among seedlings originating from the 1942 poor seed than from the 1943 good seed. Spergon-treated seed of both years gave lowest incidence of diseased and abnormal seedlings.

In regard to yield it will be noted that the differences between treatments varied more widely in the case of the 1942 seed than in that of the seed produced in 1943, and that, whereas Spergon treatment gave the highest yield with 1942 seed, Fermate treatment gave the highest yield with 1943 seed. When the data for the two different lots of seeds were subjected to the regular analysis of variance in which the check was considered as a treatment, only the difference in yield arising from the treatment of the 1942 poor seed with Spergon was statistically significant.

It would seem from the data for 1942 poor seed (Table 3) that there is a parallelism, if not a correlation, between emergence and yield. Examining the data recorded for the 1943 good seed, it is to be noted, however, that such a correlation does not exist. For example, emergence from Spergon-treated seed was 9 per cent higher than that from nontreated check, yet yield from the latter was higher. The question of emergence in relation to yield will be dealt with more fully in a later section.

EFFECT OF RATE OF PLANTING ON YIELD AND DISEASE

An experiment was planned in 1944 for the purpose of finding (a) a planting rate that might give optimum yield for the particular type of soil on which the tests were being carried out and (b) if density of plants in the row might be correlated with the incidence, spread, and severity of disease. To avoid disparity in yield that might arise from lack of uniformity in the seed, the latter was selected by hand-sorting from a lot (1943 A. K. Harrow) furnished by the Harrow Experimental Station. This selected healthy seed was planted May 18, at 4 different rates, namely, 20, 30, 40, and 50 lb. per acre. On June 8, three weeks after planting, emergence counts were made, and on October 16, the 5-replicated, randomized plots comprising the experiment were harvested and yield results taken immediately after threshing. Emergence and yield data are summarized in table 4.

TABLE 4.—*Emergence and yield in relation to difference in planting rate of selected healthy A. K. Harrow soybean seed*

Planting rate (lb. per acre)	Emergence	Yield		
		Pounds		Bu. per acre
		Total	Av.	
20	878	25.5	6.3	26.5
30	1304	27.4	6.8	28.5
40	1717	25.5	6.3	26.5
50	2145	24.6	6.1	25.5
	6044	103.0		

As might be expected, emergence was very closely correlated with rate of planting. It will be noted that (1) the highest yield was obtained from a planting rate of 30 lb. per acre, (2) yields from planting rates of 20 and 40 lb. per acre were equal, and (3) the lowest yield was obtained from a planting rate of 50 lb. per acre. When the data were subjected to the regular analysis of variance it was found that the differences in yield were not statistically significant. The important point, however, is the indication of what an important rôle some factor, probably competition, must play in modifying yield. In table 4, it will be noted that the 1304 seedlings derived from the 30-lb.-per-acre planting rate, yielded 27.4 lb. of seed, whereas the 2145 seedlings derived from the 50-lb.-per-acre rate, yielded only 24.6 lb. It would seem then, that from contiguous plots of ground of equal size and of corresponding soil type, planted with a given lot of seed, yields will be practically equal regardless of relatively wide differences in planting rates. Furthermore, it will be noted that from the 6044 seedlings that developed from the 1943 selected healthy seed, the yield of 103 lb. was not appreciably more—and certainly not significantly more—than the 102.6-lb. yield (See table 3) produced by the 1599 seedlings that originated from the 1942 poor seed. From the foregoing and other examples that the data would provide, it would appear that yield from exactly the same area (approximate 1/15 acre) was only slightly affected by treatment, selection, viability, or disease potentiality of the seed.

TREATMENT OF COMMERCIAL SEED LOTS IN 1945

To confirm previous findings or to modify them, if necessary, a further experiment in seed treatment was conducted in 1945. With few exceptions, soybeans harvested in the fall of 1944 appeared to be as healthy as those of 1943 that had matured under similar favorable conditions, though tests showed their germinability (82.4 per cent) to be slightly below that of the 1943 seed (87.2 per cent). On May 24, seed of the variety A. K. Harrow, produced by the Harrow Experimental Station in 1944, and very fairly representative of commercial seed being offered for sale in the district in the spring of 1945, was planted following treatment with Spergon (3 oz.), Arasan (2 oz.) and Fermate (2 oz.) on the site of the 1944 experiment with the 1942 seed. In the new experiment the length of the rows was reduced to 30 ft. and the rate of planting to 30 lb. per acre. On June 7, fourteen days after planting, the seedlings were breaking ground. The previous year, the seedlings from 1942 seed broke ground in 9 days. The delay in 1945 was due no doubt to the occurrence of a 5-day period of cold, wet weather. As in previous experiments, notes on emergence of seedlings and on incidence of disease and abnormality among seedlings were taken three weeks after planting, and at the end of the season yields were determined. Data acquired from 1944 high-quality seed and 1943 high-quality seed are summarized in table 5. Since length of row and rate of planting are different in the two experiments, results in table 5 are expressed on a percentage basis so that equitable comparison can be made.

Considering first the results obtained with the 1944 seed, table 5 shows that Fermate-, Arasan-, and Spergon-treated seed gave higher emergence than nontreated seed and that there was no significant difference between the effectiveness of the treatments with the three protectants. Incidence of disease and abnormality was highest among seedlings developing from nontreated seed and lowest among those originating from Spergon-treated seed. Fermate-treated seed gave the highest yield with Spergon- and Arasan-treated and nontreated following in the order mentioned. None of the differences between was found to be statistically significant.

comparing the results of the two experiments, it will be noted that emergence from 1943 treated and nontreated seed was higher than from similarly treated (or nontreated) 1944 seed. Incidence of disease and abnormality was (a) higher among seedlings from the 1944 seed than from the 1943 seed, (b) highest in both years from nontreated seed, and (c) lowest in

TABLE 5.—*Comparison of result of treatment of high quality commercial soybean seed, variety A. K. Harrow, produced 1943 and 1944*

Treatment	Percentage				Yield (bu. per acre)	
	Emergence		Incidence of disease and abnormality			
	1943	1944	1943	1944	1943	1944
Fer	85.4	80.0	3.9	12.0	27.3	27.2
Ara	85.1	79.6	6.5	13.9	26.8	24.9
Spe	95.2	78.6	1.3	9.4	26.7	27.1
Ch	85.8	62.8	6.4	25.4	27.1	24.9

both years from Spergon-treated seed. It is possible only to surmise as to the reason for the lower emergence of seedlings and the higher incidence of disease among seedlings from the 1944 seed. As pointed out, a 5-day period of cold, wet weather intervened between the planting and emergence of the 1944 seed, whereas in the case of the 1943 seed the weather was continuously favorable and the seedlings broke ground in 9 days. It seems reasonable to suppose that as the result of the 5-day exposure to cold, wet soil, some of the 1944 seeds failed to germinate while others that did germinate were destroyed by pre-emergence damping-off. If unfavorable environmental conditions were the cause of the differences observed, then it appears that under such circumstances the efficacy of seed treatment tends to be appreciably reduced.

In both years it was found that yield from treated seed was in no case significantly higher than from nontreated seed. In both years, too, identical treatments resulted in closely similar yields. This might occasion some surprise because emergence was lower from the 1944 than from the 1943 seed and at the same time incidence of disease and abnormality was appreciably higher from the former than from the latter. These facts not only confirm

but add emphasis to the comments made earlier in the present paper that, though stands of plants may differ widely numerically in the earlier part of the growing season, they tend to y ... the same. Thus, in any consideration of factors modifying yield, the ... soybean plant to adapt itself to widely-varying degrees of compe... must be regarded as important.

TREATMENT OF ETCHED AND CRACKED SEED

As a probable result of the highly favorable weather conditions that pertained in 1943 and 1944, the soybean ... produced in the district in those two years was virtually free from disease. In a critical examination of the 1944 seed (i.e., Harrow Experimental Station A. K. Harrow) it was... however, that such seed could be separated into different categories, at least two of which, (a) seed with cracked coat[*] and (b) seed with etched[7] or pitted coat, might, it was thought, have so... pathological significance. The seed in question had been harvested with ... mercial type, self-propelled

TABLE 6.—*Qualitative and quantitative "..."* ... *of combined and threshed 1944 A. K. Harrow soybean seed*

Seed harvested by	Categories, in per cent				
	Healthy	Etched or pitted coat	Cracked coat	Diseased	Broken seed and trash
Thresher[a]	78.25	17.44	1.13	0.51	
Combine[b]	62.20	13.26	9.73	0.46	

[a] J. L. Jones, No. 4, pea and bean thresher.
[b] Commercial type, self-propelled combine.

combine. When seed of the same variety that had been grown in the laboratory plots, but which had been threshed with a pea and bean thresher, was examined, it was found that while it, too, showed a relatively high proportion of etched seed, seeds with cracked coats were relatively rare. The evidence suggested that harvesting by combine must have resulted in appreciably heavy seed-coat injury. To investigate the matter further, commercial samples of soybeans (all variety A. K. Harrow) known to have been harvested with a combine were obtained. A cursory examination showed that in every sample, seeds with cracked coats could be readily found. These samples from outside sources together with some of the Harrow Station seed were then thoroughly mixed and from the mixture a 4-pound composite sample was taken. A 4-pound lot was also obtained from the seed produced in the laboratory plots. The two lots were carefully examined under the dissecting microscope and segregated by hand-sorting into the categories shown in table 6.

Two interesting and significant points were: (a) the extremely low and the relatively high percentage of diseased and healthy seed, respectively, in

[7] The term etched seed is used to describe seed that showed lighter colored surface markings which closely resembled the tracery of a filigree pattern and with which coincided a more or less roughened seed coat.

both threshed and combined seed, and (b) the much higher percentage of trash and seeds with cracked coat in the combined than in the threshed seed.

Since in the case of the etched seed as well as in that of the seed with the cracked coat the surface was either roughened or definitely broken and might, therefore, be expected to offer a "lodging-place" for pathogenic organisms, it was thought that these two categories might constitute highly suitable material for a seed-treatment test. Consequently, sufficient etched and cracked-coat seed, as well as healthy seed to serve as check, was obtained to plant duplicate, 30-ft. rows in the randomized, 5-replicate design adopted for all of the experiments. One of the duplicate rows in each replicate was planted with Spergon-treated seed (3 oz. per bu.), the other with nontreated seed. Data as to emergence of seedlings, incidence of disease, and final yield are recorded in table 7.

TABLE 7.—*Results from treated and nontreated components of 1944 commercial A. K. Harrow soybean seed*

Category	Emergence		Incidence of disease and abnormality, in per cent		Average yield (bu. per acre)		
	Spergon	Check	Spergon	Check	Spergon	Check	Difference due to treatment
Etched	570	513	7.5	15.0	27.4	25.5	+1.8
Cracked-coat	529	366	19.6	35.7	25.8	21.5	+4.3 •
Healthy	695	541	4.7	17.3	26.8	25.1	+1.6

• Significant at 5 per cent level.

Treatment with Spergon increased emergence from seeds in all three categories and correspondingly reduced incidence of disease and abnormality. In the case of both treated and nontreated seed, emergence was highest from healthy seed, intermediate from etched seed, and lowest from cracked-coat seed; whereas incidence of disease and abnormality was lowest for healthy seed, intermediate for etched seed, and highest for cracked-coat seed. Furthermore, treatment with Spergon increased the yield in the case of each component. When, however, the difference between the two means of each component was compared by the *t* test (8), only that between treated and nontreated, cracked-coat seed was found to be statistically significant. Since the seeds in this category were the most doubtful from the standpoint of germinability and seemingly the most likely to harbor pathogenic organisms, the results indicating an increase of 4.3 bu. per acre due to treatment with Spergon are regarded as being of considerable importance. In southwestern Ontario, most soybeans are harvested with combine. The latter are not always carefully adjusted and operated and as a result an appreciable fraction of the crop may be liable to injury, the degree and amount of which will probably vary with the moisture content of the soybeans at time of harvest.

The results of this experiment furnish an additional example of the lack of correlation between number of plants in early stands and final yield. Referring to table 7, it will be noted that the emergence of Spergon-treated healthy seed exceeded that of nontreated healthy seed by 154 seedlings, yet the difference in yield from identically the same lengths of row, planted originally with the same numbers of seeds, was not statistically significant.

INCIDENCE OF BROWN SPOT IN DIFFERENT VARIETIES IN 1945

The rapidly expanding acreage of soybeans in southwestern Ontario has accelerated the introduction of new varieties and selections. It was felt that before these should become established information should be obtained as to their susceptibility or resistance to the diseases prevalent in the district. With this in view, seed of the varieties Richland, Lincoln, Earlyana, and Harman, and of a Harrow Experimental Station selection, designated as Harrow A,[s] were planted in a 5-replicate, randomized design on May 25, 1945. The plots were kept under critical observation throughout the grow-

TABLE 8.—*Relative incidence of brown spot infection on different varieties of soybeans*

Varieties	Number of plants examined	Percentage of infected plants
Earlyana	633	89.5
Lincoln	682	67.7
Richland	432	66.6
Harman	562	39.6
Harrow A	349	37.5

ing season. With the exception of brown spot caused by *Septoria glycines*, the plots remained virtually free from disease. Brown spot was first noted in the varietal plots on July 3. The typical reddish brown spots were especially noticeable on the pair of unifoliate or first true leaves. Casual examination of the different varieties suggested differences in susceptibility and resistance. Counts of the unifoliate leaves with infection on July 7 are recorded in table 8.

On a basis of their relative susceptibility to brown spot, the varieties Harrow A and Harman would be classed as moderately resistant, Richland and Lincoln intermediate in susceptibility, and Earlyana highly susceptible. Not only were more leaves of the variety Earlyana infected than those of the other varieties, but they were more severely infected. Even on Earlyana, however, infection spread beyond the first pair of unifoliate leaves to only a few of the lower trifoliate leaves.

The unifoliate leaves of the variety A. K. Harrow, originating from seed treated with Arasan, Fermate, and Spergon, respectively, were no less seriously infected than those of plants developing from nontreated seed, thus

[s] For seed of this selection and of the varieties mentioned, the writers are indebted to Mr. C. Owen, Dominion Experimental Station, Harrow, Ontario.

indicating lack of correlation between incidence of brown spot and seed treatment.

DISCUSSION

The results obtained in the present investigations are in close agreement with those reported more recently by several workers, including Melhus *et al.* (18), Porter (22), Koehler (14), and Allington *et al.* (1), all of whom have found that although seed treatment might give increased stands over non-treated checks, in general, yields remained unaffected. These investigators for the most part doubt that seed treatment can be recommended except in a year following a season in which the seed has undergone severe weather damage, or when, for some reason, it might be desired to "stretch" seed by sowing less than the amount required under normal circumstances. With the above, the present writers are in accord except that with the evidence of their own experiments to lend added weight, they would express themselves more positively as to the advisability of treating the poor seed that is produced in "bad" years. They also feel that when, as the result of being harvested with a combine either unsuitable as to type or not carefully enough operated or adjusted in relation especially to existing weather conditions (too many instances of which have been encountered in Ontario), soybeans have an appreciably high percentage of seeds with a cracked coat, benefits will accrue from seed treatment. The situation over the next few years would seem to resolve itself into one of educating the grower to the point where by visual inspection he can decide for himself whether seed should be treated or not.

Of interest in the present investigations were the repeated demonstrations of remarkable uniformity of yield from plots of equal size despite wide differences in the number of plants involved. For example, it was shown that from the initial stand of seedlings from 1942 poor seed the yield was not appreciably less than that produced by almost four times as many seedlings originating from specially selected healthy 1943 seed in exactly the same length of row. Other examples similar to the above were encountered, and, considering them altogether, they provided substantiation for the statement made earlier in the present paper to the effect that, within reasonable limits, yields from contiguous plots of ground will tend to be affected only slightly by rate of planting, treatment, selection, germinability, or disease potentiality of the seed. Indeed, a new appreciation was gained not only of the capability of the soybean plant to adapt itself and respond to widely varying degrees of competition but also of the importance of this latter factor in relation to yield. With other than seed of very low germinability or of high disease potentiality, it would seem that optimum planting rate—with due consideration for soil type—might be as important as almost any other factor in obtaining maximum yield. It is noteworthy that Probst (23) who recently studied the effect of plant spacing of soybeans on yield, reported that "the least difference in yield, and usually the highest yield, was obtained

when the plants were spaced 2 or 3 inches apart." Certainly, in seed treatment experiments with soybeans, increase in early stand of plants as the result of treatment should not be regarded as necessarily indicating corresponding increase in yield.

SUMMARY

In a 3-year series of experiments (randomized, 5-replicate design), the efficacy of Spergon, Arasan, and Fermate has been tested on seed lots of the variety A. K. Harrow differing widely as to germinability, disease potentiality, and extent of seed-coat injury. With poor quality, weather-damaged seed, such as that produced in 1942, and with the cracked-coat fraction (resulting from combine injury) of an otherwise high-quality seed, such as that produced in 1944, treatment with Spergon increased emergence and yield. These were the only instances, however, in which increases in early stands of plants as the result of seed treatment were correlated with statistically significant increases in yield.

When selected healthy seed was planted at the rates of 20, 30, 40, and 50 lb. per acre, emergence of seedlings was closely correlated with rate of planting but differences in yield were not statistically significant. The results of this and other tests having shown a complete lack of correlation between stands of plants, differing numerically by a ratio almost as high as 4:1, and yield, it was concluded that the increase in early stand of plants as the result of treatment should not be regarded as necessarily indicating a corresponding increase in yield.

In a varietal resistance test, Earlyana was most susceptible, Lincoln and Richland were intermediate in susceptibility, and Harman and Harrow A (a promising unnamed selection) were most resistant to brown spot caused by *Septoria glycines* Hemmi, but the incidence of brown spot was in no way correlated with either treated or nontreated seed.

DOMINION LABORATORY OF PLANT PATHOLOGY,
    HARROW, ONTARIO, CANADA.

LITERATURE CITED

1. ALLINGTON, W. B., G. C. KENT, I. W. TERVET, and BENJAMIN KOEHLER. Results of the uniform soybean seed treatment tests in 1944. U. S. Dept. Agr., Pl. Dis. Rptr. Supp. 159: 220–224. 1945.
2. ANONYMOUS. Victory Mills Ltd. The Soybean Digest 5(8): 9. 1945.
3. BARTHOLOMEW, R. P. Seed treatment with plant hormones in crop production. Arkansas Agr. Exp. Sta. Bul. 444. 1944.
4. CONNERS, I. L., and D. B. O. SAVILE. *In* Ann. Repts. Canadian Plant Dis. Surv. 22: 29–30, 1942; 23: 29–32, 1943; 24: 30–33, 1944.
5. DAVEY, R. H. Further evidence of the fungicidal value of Spergon. U. S. Dept. Agr., Pl. Dis. Rptr. 26: 162–163. 1942.
6. DIMMOCK, F. Soybean growing in Canada. The Soybean Digest 5(8): 8–9. 1945.
7. DRAYTON, F. L. A summary of the prevalence of plant diseases in the Dominion of Canada 1920–1924. Dom. Can. Dept. Agr. Bul. 71, New Series. 1926.
8. HAYES, H. K., and F. R. IMMER. Methods of plant breeding. Ed. 1, 432 pp., McGraw-Hill Book Co., New York, N. Y. 1942.
9. HEUBERGER, J. W., and T. F. MANNS. Effect of organic and inorganic seed treatments on rate of emergence, stand and yield of soybeans. (Abstr.) Phytopath. 33: 1113. 1943.

10. ——————, and ——————. Effect of organic and inorganic seed treatments on rate of emergence, stand and yield of soybeans. Delaware Agr. Exp. Sta. Pamphlet No. 11. 1944. (Mimeographed.)
11. JOHNSON, H. W., and B. KOEHLER. Soybean diseases and their control. U. S. Dept. Agr. Farmers' Bul. 1937. 1943.
12. KIESSELBACH, T. A. Crop response to hormone seed treatments. Jour. Amer. Soc. Agron. 35: 321–331. 1943.
13. KOEHLER, B. Soybean diseases. Mimeographed leaflet prepared at the request of the War Service Committee of the Northern Mississippi Valley plant pathologists. 1943.
14. ——————. 1943 results of uniform seed treatment tests on soybeans. U. S. Dept. Agr., Pl. Dis. Rptr. Suppl. 145: 76–79. 1944.
15. ——————. New developments in soybean disease studies. The Soybean Digest 4(8): 6–7. 1944.
16. LEHMAN, S. G. Soybean diseases. In 51st Ann. Rept. North Carolina Agr. Exp. Sta., 1928, pp. 59–61. 1929.
17. McROSTIE, G. P., and J. LAUGHLAND. Soybeans in Ontario. Ontario Dept. Agr. Bul. 407. 1940.
18. MELHUS, I. E., J. N. MARTIN, and H. C. MURPHY. The influence of pythiaceous and other fungi on seedling stands of legumes and other crops. Rept. Iowa Agr. Exp. Sta. 1943–44, Part I, p. 157. 1944.
19. Ontario Monthly Crop Report. Ontario Dept. Agr., Bul. 300. 1943.
20. Ontario Monthly Crop Report. Ontario Dept. Agr., Bul. 311. 1944.
21. PETTY, M. A. Soybean diseases on the eastern shore of Maryland. Peninsular Hort. Soc. (Del.) Trans. 57: 58–62. 1943.
22. PORTER, R. H. Soybean seed treatments. U. S. Dept. Agr., Pl. Dis. Rptr. Suppl. 145: 22–25. 1944.
23. PROBST, A. H. Influence of spacing on yield and other characters in soybeans. Jour. Amer. Soc. Agron. 27: 549–554. 1945.
24. STODDARD, D. L. Seed treatment. In Report of the Federal Exp. Sta. in Puerto Rico 1944, pp. 28–29. 1945.
25. TERVET, I. W. Soybean diseases in Minnesota. U. S. Dept. Agr., Pl. Dis. Rptr., 27: 135–138. 1943.
26. ——————. Molds injurious to soybean seed. Minnesota Farm and Home Science 1: 13–14. 1943.
27. ——————. The influence of fungi on storage, on seed viability and seedling vigor of soybeans. Phytopath. 35: 3–15. 1945.
28. WOLF, F. A., and S. G. LEHMAN. Soybean diseases. In 48th Ann. Rept. North Carolina Agr. Exp. Sta. 1925, pp. 31–32. 1926.

ORGANIC COMPOUNDS FOR CONTROL OF TOBACCO BLUE MOLD

T. W. GRAHAM, E. E. CLAYTON, J. G. GAINES,
T. E. SMITH, AND F. A. TODD[1]

INTRODUCTION

The blue-mold disease of tobacco (*Nicotiana tabacum* L.) caused by *Peronospora tabacina* Adam is of major importance in the tobacco growing areas of the United States, particularly in the southeastern states of Georgia, North and South Carolina, and Virginia. The disease is more severe in cool, moist weather, which frequently occurs in the early spring months when seedling tobacco plants are small and succulent. This coincidence is favorable to epidemic outbreaks which have recurred each year since 1931, with consequent delay in transplanting, and often serious plant shortages. The continued destructiveness of blue mold has stimulated much research on methods of control by several State and Federal agencies. The two treatments that were first developed and introduced commercially were copper oxide-oil spray (2) and fumigation treatment with paradichlorobenzene (3). Further investigation showed that organic fungicide sprays and dusts were effective also, particularly certain of the salicylate compounds as well as Fermate and other organic fungicides (1, 4, 7, 8).

Tobacco growers generally have not adopted these control measures, but instead have depended on increased yardage of plant beds and other cultural practices. Such methods have not been effective under epidemic conditions of blue mold, as was clearly demonstrated in North Carolina in 1945, when over 90 per cent of the crop of some entire counties was set 10 to 20 days later than normal or was set from plants transported as far as 200 miles. Therefore, the need exists for an effective treatment which is easy to apply and inexpensive enough to adopt as a part of the general operations of the grower. This paper reports further results in the continuing search for less expensive fungicides, more efficient formulations, and more convenient methods of application.

EXPERIMENTAL METHODS

Both greenhouse and outside plant-bed tests were used in evaluating the fungicides. Results obtained in the greenhouse were regarded as preliminary, inasmuch as plant-bed conditions could not be fully duplicated. Blue mold was easily maintained and very active in the greenhouse during the winter months. Unprotected check plants usually had 60 to 100 per cent of leaf area killed.

The disease in plant beds was more severe in cool, cloudy, or rainy

weather, and these conditions were frequent enough during the seasons of 1940, 1941, 1943, 1944, 1945, and 1946 to give critical tests of the fungicides used. Conditions were unfavorable for the development of blue mold during 1942, and the results were not regarded as critical. These data are not included.

Plants were grown in 4- or 6-inch pots for greenhouse experiments. Uniform stands of 6 to 12 plants were used in 4-inch pots, and 15 to 20 plants in 6-inch pots. Treatments were begun on plants four to six weeks old, when leaves were $\frac{1}{2}$ to $\frac{3}{4}$ inch in diameter. Two to three pots were used in preliminary experiments, and five to ten pots in critical tests. Sprays and dusts were applied twice weekly with a small hand sprayer or duster during a period of 3 to 4 weeks. Plants were inoculated after the third or fourth treatment and placed under a moist muslin tent.[2] Disease severity was determined 1 to 3 weeks after the final treatment, by counts of the dead and partly killed leaves. Injured leaves were placed in 3 classes of disease severity, i.e., entire leaf area dead, approximately one-half of leaf area dead, and approximately one-fourth of leaf area dead. Percentage of leaves in each class was determined from total number of leaves exposed to infection. The percentages in class 1 were multiplied by 1.0, those in class 2 by 0.5, and those in class 3 by 0.25; and the sum of these values was used as the disease index in which 0 indicates no symptoms and 100 complete defoliation.

Plant-bed treatments were started when tobacco leaves were one-half to one inch in diameter. Plants usually reach this stage the last week in March, or the first week in April, at the Experiment Station in Florence, South Carolina. Dusts and sprays were applied twice weekly, which was sufficient to keep new growth covered with fungicide. Plants were inoculated after the third or fourth treatment, and also were subject to natural infection by wind-blown spores from adjacent untreated beds. Disease readings were taken 1 to 3 weeks following final treatment.

EXPERIMENTS WITH SPRAYS

From 1940 through 1946 numerous spray and dust experiments were completed at the Florence, South Carolina, Experiment Station. More than 250 materials were tested in the greenhouse in the course of approximately 75 separate spray or dust experiments. Plant-bed tests also were conducted each year in which all materials were included that showed promise in the greenhouse. In addition to this, there were extensive supplementary plant-bed tests each year at the Georgia Coastal Plain Experiment Station, at Tifton, Georgia, and at the Tobacco Branch Experiment Stations at Oxford and at McCullers, North Carolina. A large mass of experimental data was obtained, obviously much too detailed for complete presentation here. Results are therefore given only from the most significant or the most illus-

---

[2] A muslin tent was placed over a portion of the greenhouse bench, above which a spray nozzle was suspended. Relative humidity was 100 per cent when spray was running. Inoculated plants were kept under the tent for 1 to 2 weeks to allow for infection and abundant sporulation by the blue-mold fungus.

trative experiments.   The salicylate compounds proved to be particularly effective.   Clayton *et al.* (4) reported the value of bismuth subsalicylate and benzyl salicylate, the latter used with cottonseed oil.   Brief reports were also published by the senior writer (7, 8) on the effect of various salicylates and certain other organics used in both non-oil sprays and in dust preparations. In the earlier greenhouse and plant-bed tests copper oxide–oil was used for comparison.   The degree of protection given by some of the salicylates used without oil was higher than that for copper oxide–oil.   Materials were dissolved or suspended in suitable solvents from which dilutions were made with

TABLE 1.—*Blue-mold control in the greenhouse with salicylate sprays*

Material	Amt. per 100 gal. of spray	Disease index[a]				
		Plot 1	Plot 2	Plot 3	Plot 4	Average
	Lb.					
Acetylsalicylic acid	0.25	8	5	8	13	8.5[*b]
Benzyl salicylate	1.0	18	7	17	12	13.5
Betanaphthyl salicylate	0.25	11	10	11	18	12.5
Bismuth subsalicylate	1.5	0	0	0	0	0 [*]
Copper oxide–oil	1.0–1.0[c]	22	25	17	12	19.0
Isoamyl salicylate	1.0	11	7	13	5	9.0[*]
Methyl salicylate	1.0	24	25	37	30	29.0
N-butyl salicylate	0.5	23	6	10	15	13.5
Phenyl salicylate	0.5	30	33	38	28	32.2
Salicylaldehyde	1.0	16	15	36	27	23.5
Salicylamide	1.5	20	8	9	15	13.0
Salicylic acid	0.5	6	5	13	15	9.7[*]
Sodium salicylate	0.5	17	12	24	15	17.0
Zinc salicylate	0.25	25	30	27	23	26.2
Control (not treated)	....	87	95	72	78	83.0
Difference required for significance at 5 per cent level					....	8.25
Difference required for significance at 1 per cent level						11.02

[a] The disease index used here is a percentage figure, which is the sum of the disease values assigned to leaves in 3 classes of blue mold severity; *i.e.*, percentage with entire leaf area dead × 1, plus percentage with ⅓ of leaf area dead × 0.5, plus percentage with ¼ of leaf area dead × 0.25.   100 equals complete defoliation and 0 equals no symptom.

[b] Values significantly lower than the value for copper oxide–oil at 5 per cent level are indicated by an asterisk (*).

[c] One lb. copper oxide and 1 gal. cottonseed oil for 100 gal. of spray.

water after emulsifying with 0.01 per cent B–1956 emulsifier.[3]   Some 24 salicylates have been tested.

Table 1 presents results from a typical greenhouse experiment in which 13 salicylates were included.   Bismuth subsalicylate was outstanding, and has been consistently so in both greenhouse and plant beds.   (Fig. 1.)   Good results were obtained also with acetylsalicylic acid, isoamyl salicylate, and salicylic acid.   Each was more effective than copper oxide–oil.   The margin between fungicidal and phytotoxic levels was relatively narrow with salicylic acid, acetylsalicylic acid, and betanaphthyl salicylate.   Other salicylates tested but not listed in table 1 were: ethyl salicylate, isopropyl salicylate, salicylaldoxime, sodium hydrogen sulphosalicylate, and sulphosalicylic acid.

[3] A phthalic anhydride glycerol alkyl resin.

5-aminosalicylic acid hydrochloride, 3,5-dinitrosalicylic acid, salicylidene-acetamide, and thiosalicylic acid. This latter group gave varying degrees of slight to moderate protection from blue mold. None gave such consistently high protection as to justify continued tests with them. While results in table 1 indicate the fungicidal value of the respective salicylates, these and other greenhouse results were not always in close agreement with those obtained in plant beds. Results here, therefore, are not intended to imply the value of these materials in plant beds. For example, in plant-bed tests at Tifton, Georgia, bismuth subsalicylate and zinc and benzyl salicylates were rated highest, and acetylsalicylic acid was somewhat less effective than indicated in table 1.
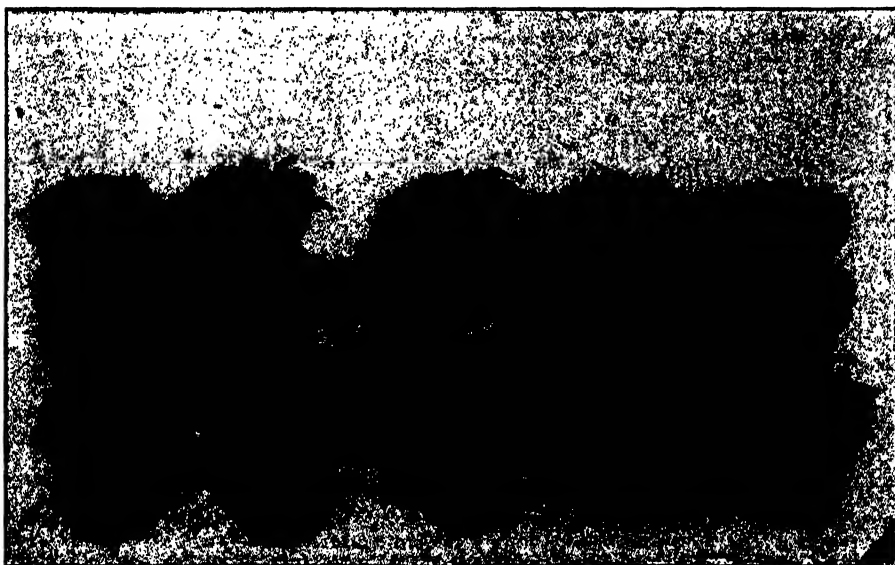
FIG. 1. Control of blue mold with salicylate sprays. Vertical rows, left to right: untreated, disease index 83; zinc salicylate, disease index 26; bismuth subsalicylate, disease index 0; betanaphthyl salicylate, disease index 13; benzyl salicylate, disease index 14.

Investigations by workers in recent years demonstrated the fungicidal value of numerous recently synthesized organic compounds. Tisdale and Williams (11) originally described the fungicidal properties of certain derivatives of dithiocarbamic acid. One of these, Fermate, (ferric dimethyldithiocarbamate) has since been proven effective by various workers (12). Disodium ethylene bisdithiocarbamate (Dithane) is another derivative which is water-soluble and highly fungicidal (5). Other derivatives include zinc dimethyldithiocarbamate (Zerlate) and tetramethyl thiuramdisulphide (Thiosan and Arasan). The latter is used as a seed and turf fungicide. Tetrachloro-parabenzoquinone (Spergon) is chemically unrelated to the above compounds, but is widely used as a seed protectant. The following are other recently studied organics that have been proven fungicidal: 2,3-dichloro-1,4-naphthoquinone (U.S.R. No. 604), 2,2-dihydroxy-5,5 dichloro-

phenylmethane (Preventol GD), and phenanthra quinone. All of these and numerous others were used against blue mold. The great majority were not effective, some gave slight to moderate protection, and a few were very effective.

Table 2 presents a summary of results from greenhouse and plant-bed

TABLE 2.—*Amount of blue mold in greenhouse and plant-bed tests of organic and other spray materials, 1941–45*

Material	Greenhouse			Plant beds		
	Amt. per 100 gal.	No. of tests	Average disease index	Amt. per 100 gal.	No. of tests	Average disease index
	Lb.			*Lb.*		
Bismuth subsalicylate	1.5	1	1	1.5	10	4
Benzyl salicylate	1.0	1	9	2.0	1	2
Benzoyl peroxide	1.0ª	4	7	3.0	1	0
Dithane (H.E. 175)	1.0	1	2	2.0	3	3
Copper oxide–oil	1.0	33	19	1.0	24	11
Fermate	4.0	2	0	4.0	5	8
Salicylic acid	0.5	2	10	...b	....	.
Zinc salicylate	1.0	5	5	0.25	2	12
Benzoic acid	1.0	2	27	2.0	1	0
Dithane (D–14)	..c	1	4		1	8
Spergon	1.5	1	6	4.0	3	8
Thiosan	4.0	2	1	4.0	3	50
p-Toluenesulphonylamide	1.0	2	12	1.0	1	41
Diphenyl-pp'-disulphonic acid	1.0	1	8	1.0	1	48
p-Hydroxyazobenzene	0.25	1	6	0.25	1	35
Guaiacol benzoate	2.0	1	2	1.5	3	24
U.S.R. No. 604	1.0	1	0	2.0	1	54
Zinc dimethyldithiocarbamate	1.0	1	2	2.0	1	23
4-Chlorophenyl-4-toluene sulphate	1.5	1	9	......	......	.....
2,5-Diaminotoluene sulphate	0.125	1	13	......	......	...
o-Phthallic acid	0.5	1	13	......	.....	......
p-Cresyl benzoate	3.0	1	15	......	.. ..	......
Pentachlorophenol	0.0625	1	17	......	......	......
Benzoyl chloride	1.0	2	23	......	......	......
Diethylanalin	3.0	1	25	......	....	......
Isoamyl benzoate	3.0	1	25	......	..	......
Control (not treated)	..	31	78	.....	26	60

ª Only those formulations that gave best control are listed. Benzoyl peroxide, and some of the other materials, were used in varying rates only slightly lower or higher than those listed, so that the total number of tests was greater than that shown in the table, but in each case the final disease index was only slightly different. Benzoyl peroxide is a strong oxidizing agent and is potentially explosive.

b No plant-bed results were obtained at Florence, S. C., but in tests at Tifton, Ga., salicylic acid was about equal to zinc salicylate.

c According to directions of manufacturer, 2 quarts per 100 gallons, plus 1 lb. zinc sulphate and ½ lb. hydrated lime.

experiments with some of the best organic and other materials during 1941 through 1945. Materials were placed in three groups according to the degree of blue-mold control in plant beds as follows: (1) equal to bismuth subsalicylate; (2) equal to copper oxide–oil; (3) less effective than copper oxide–oil, but with some fungicidal value. Those approaching the control value

of bismuth subsalicylate were most effective. Benzyl salicylate was most extensively tested and gave consistently good results. Benzoyl peroxide and Dithane (H.E. 175) are rated high in table 2, but results were from limited plant-bed tests and these materials have not been sufficiently tested on a large scale to rate them among the best. Those materials in group 2 which gave approximately the same control as copper oxide–oil were Fermate, salicylic acid, and zinc salicylate. Fermate was most exhaustively tested and proved effective under varying conditions. Zinc salicylate was most useful in combinations with other fungicides as described later in experiments with dust materials. A similar effect was found in sprays when zinc salicylate and salicylic acid were combined with Fermate and with Thiosan. A full report of the results with spray combinations will be presented in later publications. Dithane D–14, Spergon, and Thiosan were placed in group 3 of table 2 because they were not consistently effective, although in some tests they appeared equal to Fermate and the best salicylates. Benzoic

TABLE 3.—*Amount of blue mold in tests of selected organic sprays in a plant-bed experiment at Florence, South Carolina, in 1945*

Material	Amt. per 100 gal. of spray	Disease index				
		Plot 1	Plot 2	Plot 3	Plot 4	Average
	Lb.					
Bismuth subsalicylate	1.5	7	6	6	7	6.5
U.S.R. No. 604	1.5	62	61	48	45	54.0
Dithane D–14	*	9	6	7	8	7.5
Fermate	4.0	4	6	11	7	7.0
Zinc dimethyldithiocarbamate	2.0	20	16	21	37	23.5
Control (not treated)	. ...	90	77	77	70	78.5

* According to directions of manufacturer. See table 2, footnote c.

acid, although effective at Florence, S. C., was not equal to the salicylates in tests at Tifton, Georgia. The remaining materials in table 2 were slightly to moderately active as fungicides, but were not so effective as the above. Most of those in group 3 had narrow margins between fungicidal and phytotoxic levels, and results with them did not seem to justify further exhaustive tests. Some of these were tried only in the greenhouse. Table 2 shows that while greenhouse results were usually indicative of the fungicidal value of the materials under test, the results were frequently misleading. Thiosan, zinc dimethyldithiocarbamate, and U.S.R. No. 604 gave good protection in the greenhouse, but failed to do so in plant beds. On the other hand, copper oxide–oil gave slightly better protection in plant beds than in the greenhouse.

Table 3 presents results from a comparative plant-bed test made in 1945 with a group of the better organic sprays. Spray materials, except Dithane D–14, were used with Vatsol OTC as a wetting agent at the rate of ½ pound to 100 gallons of water. Sprays were applied twice weekly during March 19 to April 13. Disease readings were taken March 30. Bismuth subsalicylate, Fermate, and Dithane D–14 were about equally effective; each gave excellent

control.   Zinc dimethyldithiocarbamate gave fair control, but U.S.R. No. 604 was relatively ineffective.

Results from spray tests and from observation among growers indicated that certain blue-mold fungicides or combinations have pronounced residual effects.   For example, many growers controlled blue mold until the end of the season and later had an outbreak after treatments were stopped, which could be attributed to missing the critical applications or stopping treatments too early.   Experience showed that bismuth subsalicylate, for example, could be used with more latitude in treating schedules than Fermate. It has been rather difficult to secure exact data on residual protection, but the following results from the McCullers, N. C., tobacco substation illustrate the residual protection offered by bismuth subsalicylate in a series of cooperative tests among tobacco growers in 1945.   Table 4 shows that the three fungi-

TABLE 4.—*Residual effect of bismuth subsalicylate spray on control of blue mold in plant-bed tests, 1945*[a]

Material	Amt. in 100 gal.	Average disease index		
		Days between last treatment and disease readings		
		0–2	7–10	14–16
	Lb.			
Copper oxide–oil	1.0	11.9	7.4	35.8
Fermate	2.0	2.7	3.8	27.9
Bismuth subsalicylate	1.5	0.6	6.6	19.4
Control	... ..	67.1	56.3	44.7

[a] Four to 9 spray applications were made in an approximate twice-weekly schedule. Results were summarized from tests at 10 bed locations in Wake County, N. C. In most locations 100 sq. yd. of bed area were treated with each fungicide.

cides compared gave about equal protection for 10 days after sprays were stopped.   The extended protection of bismuth subsalicylate was most evident when 14–16 days elapsed, as disease indices were 19.4 for this material, as compared with 27.9 and 35.8, respectively, where Fermate and copper oxide–oil were used.   These results were confirmed by observation of numerous plant-bed trials during 1941 through 1945 in Georgia and North and South Carolina.

EXPERIMENTS WITH DUSTS

Tests with dusts, started in 1942 at the Pee Dee Experiment Station, Florence, South Carolina, and later at Tifton, Georgia, and Oxford, North Carolina, have shown that some dusts are effective against blue mold.   Copper and sulphur compounds in dust preparations were of little or no value, and among the numerous materials tried only the organics gave good protection. Results in table 5 represent the relative control value of various dusting materials.   Bismuth subsalicylate was outstanding and gave excellent protection in repeated tests.   Fermate gave good control, but was somewhat less

TABLE 5.—*Amount of blue mold in greenhouse and plant-bed tests of organic dust fungicides, 1943–45*

Material[a]	Amt. per 100 lb. of dust	Greenhouse		Plant beds	
		No. of tests	Average disease index	No. of tests	Average disease index
	Lb.				
Bismuth subsalicylate	10 to 15	6	9	2	4
Dithane (H.E. 175)	5 to 10	5	3	1	5
Fermate	10 to 15	5	13	3	10
Sodium salicylate	0.5 to 1	3	12	2	11
Spergon	10	3	19	1	41
Thiosan	10	1	12	1	46
Zinc salicylate	2	4	16	1	16
Control (not treated)	..	10	84	4	52

[a] Pyrophyllite, Pyrax ABB, was used throughout as a diluent.

effective than bismuth subsalicylate (Fig. 2).[4] Results with Thiosan and Spergon were erratic, although in some tests they were effective; generally results with them were similar to those obtained in spray tests. Zinc salicylate and sodium salicylate, while effective, frequently caused plant injury. Dithane (H.E. 175) was very effective, but tests with this material were discontinued in 1945 because it was no longer available in a form suitable for dusting.[5]

Fig. 2. Control of blue mold with organic dusts. Vertical rows, left to right: Untreated, disease index 92; 2 per cent U.S.R. No. 604, disease index 26; 15 per cent Fermate, disease index 6; 10 per cent bismuth subsalicylate, disease index 0.

[4] In another comparative trial in 1946 bismuth subsalicylate was somewhat less effective than Fermate, while in all other tests the former was consistently best. This reversal of results may have been due to the special grade of bismuth subsalicylate used in 1946; however, this point has not been determined.

[5] The manufacture of this material in dry form was discontinued by the manufacturer, and was replaced by Dithane D-14, a liquid for use in sprays.

In early experiments with single materials the most effective concentrations were found. Tests with Fermate showed that 15 per cent was most satisfactory and that amounts of Fermate above this were not correspondingly better. For example, in one experiment 30 per cent Fermate was no better than 15 per, cent. Repeated tests in plant beds confirmed this, and showed that when adequate amounts of dust were applied, 15 per cent gave adequate protection (Fig. 3). Similarly, bismuth subsalicylate, Spergon and Thiosan were most effective in 10 per cent dusts. Zinc salicylate gave maximum protection at 2 per cent, but higher concentrations caused marked plant injury.
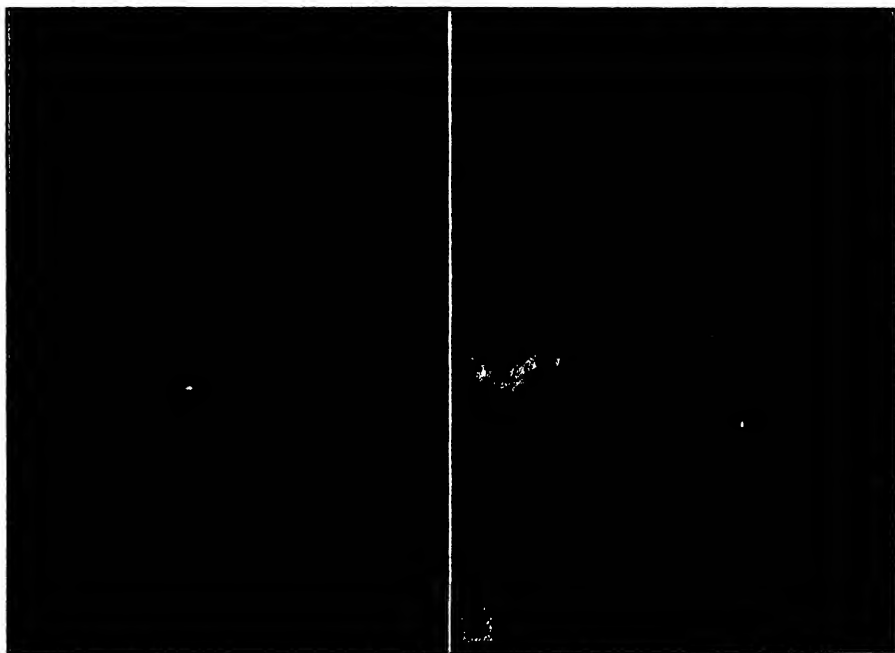
FIG. 3.   Blue mold in tobacco plant beds.   A.   Plants protected with Fermate–zinc salicylate dust.   B.   Untreated plants with severe blue-mold infection.

Table 6 presents results from a plant-bed experiment to determine the amount of dust necessary to control blue mold. Results with Fermate (15 per cent) in a test at Oxford, N. C., in 1944, showed that relatively large amounts and thorough coverage were necessary. Beds receiving 17.25 or more lb. per 100 sq. yd. developed very little blue mold and the bed receiving 11.50 lb. also remained relatively disease-free, but half this amount (5.75 lb.) was not effective. These results are in general agreement with those obtained at Florence, S. C., and at Tifton, Ga., where rates of 16 to 20 lb. per 100 sq. yd. gave adequate control.

Early experiments with dusts indicated that certain dust mixtures gave superior protection against blue mold, an effect that has been described as

TABLE 6.—*Rate of application of Fermate dust in relation to blue-mold control in plant beds[a] at Oxford, N. C., in 1944*

Amount of dust used on 100 sq. yd.				Disease index
Treatments 1 and 2	Treatment 3	Treatments 4, 5, 6, and 7	Total for 7 treatments	
Lb.	*Lb.*	*Lb.*	*Lb.*	
0.0	0.0	0.0	0.0	56.5
0.5	0.75	1.0	5.75	33.4
1.0	1.50	2.0	11.50	8.8
1.5	2.25	3.0	17.25	6.5
Fermate dust, not weighed but dusted heavily to give thorough coverage. Seven treatments applied				2.2

[a] Plots were 100 sq. yd. (5 × 20), and so arranged that one long side of each was adjacent to an untreated area. Applications were made on single plots for each rate with a Root hand-crank duster and applied in a twice-weekly schedule for a total of 7 applications. Dust formulation was 15 per cent Fermate in Pyrax ABB clay diluent.

synergism.[e] This phenomenon was observed by Clayton *et al.* (4) in copper oxide–oil spray used against blue mold. They state, "It is readily possible to show by computation that the protection provided by the combination of copper and oil is significantly greater than the sum of the protection provided by each separately." Dimond and Horsfall (6) observed a similar effect with combinations of copper and sulphur used to control potato tip burn.

The action of dust combinations against blue mold is illustrated by the following greenhouse experiment, which is representative of a series of such tests made during 1944 and 1945. A randomized block test with six single-pot replicates was used. Dusts were applied twice weekly for a total of 6 treatments. Zinc salicylate was combined with Fermate and each of these components also was applied separately in the same concentration. The concentrations used were well below those known to give effective blue-mold control. Dust applications were very light and uniform, and conditions for blue-mold development were very favorable. Table 7 shows that 1.0 per cent

TABLE 7.—*Synergistic effect of a combination of Fermate and zinc salicylate dust on control of blue mold, 1944*

Material	Amount per 100 lb. dust (lb.)	Average disease index
Fermate	1.0	98.8
Zinc salicylate	0.5	83.0
Fermate and zinc salicylate	1.0 and 0.5	45.1
Fermate	15.0	36.8
Fermate	30.0	30.6
Difference required for significance at 5 per cent level		7.8
Difference required for significance at 1 per cent level		10.64

[e] The term synergism is used here to describe the phenomenon in which the total fungicidal effect of combined materials is greater than that of the sum of the component parts used independently.

Fermate reduced blue mold only 1.2 per cent and that 0.5 per cent zinc salicylate reduced it 17.0 per cent. · A mixture of the two in the same concentration reduced blue mold 54.9 per cent, which was 36.7 per cent more than the sum of values for the single components. Both 15 per cent and 30 per cent Fermate gave only partial control (disease indices were 36.8 and 30.6, respectively) which shows that light dusting and favorable conditions for blue mold gave a critical test of Fermate and zinc salicylate at the low concentrations used.

Table 8 presents results from further experiments in plant beds during 1945 to obtain more information on the action of combined dusts. A randomized block test was arranged with 4 replicate plots, each of approximately 2 sq. yd. Treatments were applied with a small, hand, plunger-type duster in a twice-weekly schedule during March 20 to April 5. Zinc salicylate was combined with Fermate, Thiosan, and Spergon. Results demonstrated the

TABLE 8.—*Synergistic and residual effect of single and combined dusts on control of blue mold, 1945*

Material	Amt. in 100 lb. of dust	Average disease index when used	
		Alone	Combined with 2 lb. zinc salicylate per 100 lb. of dust
	Lb.		
Bismuth subsalicylate	10.0	7.2	....
Zinc salicylate	2.0	16.0	.. ..
Fermate	15.0	19.2	8.0
Fermate	30.0	21.0	....
Spergon	10.0	41.7	29.2
Thiosan	10.0	46.2	8.7
Control (not treated) .	..	52.7	.. ..

Difference required for significance at 5 per cent level: 9.47
Difference required for significance at 1 per cent level: 12.79

superior value of the combined fungicides as compared with the single materials. For example, when 2 per cent zinc salicylate was added to 15 per cent Fermate, 10 per cent Thiosan, or to 10 per cent Spergon, disease indices, were respectively 8.0, 8.7, and 29.2, but when used without zinc salicylate indices were 19.2, 46.2, and 41.7, in respective order. Differences were significant in each case.

The results in table 8 also show the residual protection of these mixtures, since 21 days elapsed between the final treatment and the disease readings. During the period of treatment (March 20 to April 5) weather conditions were not favorable for active blue mold. However, beginning on April 18 a period of rainy and cloudy weather allowed blue mold to become active so that disease damage could be noted a week later. Results, therefore, are an expression of the residual protection. Bismuth subsalicylate used alone was an outstanding protectant. In earlier tests this fungicide had a marked residual effect in sprays also. The residual protection of the zinc salicylate—

Fermate mixture and the zinc salicylate–Thiosan mixture was approximately equal to that of bismuth subsalicylate. The zinc salicylate–Spergon mixture was comparatively ineffective, although it was much superior to Spergon alone.

TESTS WITH GROWERS

Further information on the practical value of dusts against blue mold was obtained in cooperative tests with tobacco growers in 1945. Several of the growers used 15 per cent Fermate in comparative tests with zinc salicylate combined with Fermate, with Spergon, and with Thiosan (Table 8). Disease control was good except at one location where severe infection had developed before treatments were started. At this location severe blue mold appeared the morning after the first treatment was applied. This indicated infection had become so general in the bed that the treatments had no opportunity to check spread of the disease. It is known that blue mold requires 6 to 10 days for sporulation to occur after plants are inoculated. In all other locations the fungicidal value was increased when zinc salicylate was added to Fermate, Thiosan, or Spergon. Fermate alone, however, gave adequate control, because four growers who used it checked blue mold completely and the remaining five growers found very slight infection that caused practically no damage. In every community where dusts were used, the beds not treated suffered severely from blue mold. In seven of the cooperative dusting experiments, treatments were begun after primary infection had appeared, yet in each case blue mold was effectively checked so that little or no damage resulted. Similarly at the Pee Dee Experiment Station, Florence, S. C., dust treatments were begun on three beds after primary areas of infection started. Secondary spread of blue mold was checked almost immediately, and the primary infections became inactive.

During 1946 a severe epidemic of blue mold in South Carolina gave an opportunity for critical tests with Fermate (15 per cent in pyrophyllite) which was sold commercially and used by many growers.[7] The writer had an opportunity to observe closely the beds of about 15 growers who used Fermate dust. Results demonstrated excellent control of blue mold. Most treated beds remained entirely free of the disease and none of them was delayed in transplanting, while nontreated beds often suffered high plant mortality (50 to 75 per cent) and there was serious delay in transplanting. Reports generally throughout the State and elsewhere have confirmed these observations.

DISCUSSION

Treatment for the control of tobacco blue mold has been simplified and made more effective through the development of organic fungicides for either spraying or dusting. Most of the organic sprays are easily mixed with water and applied directly—a simpler procedure than mixing copper oxide and emulsifying cottonseed oil separately before combining the two. Dust treat-

[7] Approximately 20 tons of Fermate dust were used by South Carolina tobacco growers in 1946.

ments are even more advantageous, as tobacco beds are frequently located so that transporting water to them is a problem.

The salicylate sprays were highly effective against blue mold. Bismuth subsalicylate and benzyl salicylate were tested extensively in commercial beds with most satisfactory results. Salicylic acid and zinc salicylate were somewhat less satisfactory, but were fungicidal when used alone and especially when used in combination with certain sprays and dusts. In addition, five other salicylates probably deserve further testing as fungicides. Fermate spray also has been exhaustively tested by growers, and its value against blue mold is well established, although it is slightly less effective than some of the salicylates. Certain derivatives of benzoic acid gave considerable protection. Benzoyl peroxide was of particular interest, as repeated trials gave good results. It appears unlikely that such a strong oxidizing agent would remain stable long enough to react directly to stop spore germination or initial infection. Some new oxidation product may have been formed which acted as a fungicide. Exhaustive trials have demonstrated that dusts were equally as effective as sprays against blue mold. Those organic toxicants that were fungicidal as sprays, and were physically suitable for dusting, proved to be fungicidal also as dusts; i.e., bismuth subsalicylate, Fermate, and zinc salicylate.

Certain combinations of fungicides gave a fungicidal effect greater than that obtained with single materials—that is, a synergistic effect. This is an important principle, and deserves further attention in disease-control practices. A synergistic combination may increase not only the fungicidal potency, but also allow the use of greatly reduced dosages. Organic fungicides appear to be particularly suitable for studies of this kind.

The residual protection provided by bismuth subsalicylate when applied as a spray or as a dust, and that of certain combined dusts containing zinc salicylate, is of both practical and theoretical interest. Its practical value is obvious. The writers saw no evidence that prolonged protection was due to more efficient coverage or better adhesion of these materials. Since adhesion was probably not a factor, it seems possible that a physiologic reaction between the salicylates and the plant tissues may have been involved. A similar phenomenon was observed by Clayton et al. (4), who pointed out that the protection provided by copper oxide–oil was not due to the same type of action usually found in sprays designed to give a protective layer of fungicide on exposed plant surfaces, and that such sprays as Bordeaux mixture were not effective against tobacco blue mold. Their suggestion was that the oil in the copper-oil spray acted in some indirect manner to promote resistance to blue mold.

SUMMARY

More than 250 organic toxicants and other materials were tested as blue-mold fungicides in the greenhouse and plant beds during 1941 through 1946 at Pee Dee Experiment Station, Florence, South Carolina. Extensive supplementary plant-bed tests were conducted also at Coastal Plain Experiment

Station, Tifton, Georgia, and the Tobacco Branch Experiment Stations at Oxford and McCullers, North Carolina. Bismuth subsalicylate was highly effective against blue mold and, in addition, benzyl salicylate, acetyl salicylate, salicylamide, salicylic acid, and sodium salicylate gave better control than copper oxide–oil. Certain derivatives of benzoic acid, especially benzoyl peroxide, were fungicidal. Fermate gave effective control in extensive experimental tests and in trials in commercial beds. In limited tests, Dithane D-14 gave satisfactory control but further testing is required for this material.

Certain organic fungicides controlled blue mold when used as dusts just as effectively as they had when used as sprays. Bismuth subsalicylate and Fermate both gave good results as dusts. Relatively large amounts of dust (17 to 20 lb. per 100 sq. yd. of bed area through the season) and thorough coverage were required. Combinations of zinc salicylate with Fermate, Thiosan, and Spergon were more effective than any of the components used alone.

Pronounced residual protection was secured by spraying or dusting with bismuth subsalicylate, or by dusting with combinations of zinc salicylate with Fermate and Thiosan.

LITERATURE CITED

1. ANDERSON, P. J.   Control of blue mold of tobacco by a new spray.   Science 86: 409. 1942.
2. CLAYTON, E. E., J. G. GAINES, T. E. SMITH, W. M. LUNN, and K. J. SHAW.   Control of blue mold (downy mildew) disease of tobacco by spraying.   U. S. Dept. Agr., Tech. Bul. 650.   1938.
3. ——————, ——————————, K. J. SHAW, T. E. SMITH, H. H. FOSTER, W. M. LUNN, and T. W. GRAHAM.   Gas treatment for control of blue mold disease of tobacco. U. S. Dept. Agr., Tech. Bul. 799.   1942.
4. ——————, T. E. SMITH, K. J. SHAW, J. G. GAINES, T. W. GRAHAM, and C. C. YEAGER, JR.   Fungicidal tests on blue mold (Peronospora tabacina) of tobacco. Jour. Agr. Res. [U.S.] 66: 261–276.   1943.
5. DIMOND, A. E., J. W. HEUBERGER, and J. G. HORSFALL.   A water soluble protectant fungicide with tenacity.   Phytopath. 33: 1095–1097.   1943.
6. ——————, and J. G. HORSFALL.   Synergism as a tool in the conservation of fungicides.   Phytopath. 34: 136–139.   1944.
7. GRAHAM, T. W.   Tobacco diseases (Blue mold).   56th Ann. Rept. South Carolina Exp. Sta. 92–95.   1944.
8. ——————.   Blue mold of tobacco.   57th Ann. Rept. South Carolina Exp. Sta. 111–114.   1945.
9. HAMILTON, J. M., D. H. PALMITER, and L. O. WEAVER.   Evaluation of Fermate for the control of apple scab and cedar-apple rust fungi (Abstr.).   Phytopath. 33: 5.   1943.
10. HENDERSON, R. G.   Studies on tobacco downy mildew in Virginia.   Va. Agr. Exp. Sta. Tech. Bul. 62.   1937.
11. TISDALE, W. H., and I. WILLIAMS.   Disinfectant.   U. S. Patent 1,972,961.   1934.
12. WILCOX, R. B.   Fermate sprays effected good control of cranberry fruit rots in New Jersey.   U. S. Dept. Agr., Pl. Dis. Rptr. 28: 34.   1944.

STABILITY OF LABILE VIRUSES IN DESICCATED TISSUE

H. H. McKinney [1]

INTRODUCTION

In most climates the atmospheric moisture varies to such an extent that the drying of mosaic leaf tissue in laboratory atmosphere is subject to extreme irregularities, especially when whole leaves, attached to or detached from the stem, are used. Under humid conditions, whole leaves give up their moisture very slowly, fermentation processes destroy the plastid pigments, and the tissues do not become crisp. During periods of dry weather, such tissue dries more rapidly, but the plastid pigments are usually destroyed during the drying process, and, when dried under either of these conditions, many viruses do not survive for more than relatively short periods.

Working with the virus of cucumber mosaic, it was found that finely cut mosaic tobacco leaves dried in the laboratory sometimes contained traces of viable virus after the tissue had reached the stage commonly regarded as dry. However, no virus was recovered when whole leaves were similarly dried.

Since it is commonly known that fermentation processes are retarded at low temperatures, and that many of the labile viruses retain their activity for fairly long periods in expressed plant juices when held at or near freezing temperatures, it seemed reasonable that methods might be devised whereby certain of the labile viruses will retain a large part of their activity in dry tissue. A brief report of these studies with cucumber-mosaic virus has been published.[2]

METHODS AND MATERIALS

Leaf tissues were used throughout the studies. Desiccation was carried out in the free atmosphere and also in desiccators, made from baking pans $1\frac{1}{2} \times 7 \times 11$ inches. Calcium chloride crystals were placed in the bottom, a wire screen ($\frac{1}{4}$-inch mesh) above the crystals supported a layer of surgical gauze and the tissue. To prevent excessive absorption of tissue juices, the gauze was lightly sprayed with a solution of paraffin and any highly volatile solvent, and the solvent was allowed to evaporate. Care was exercised to avoid clogging the meshes of the gauze. Each pan was covered with a piece of glass cut to the dimensions of the pan. The edges of the glass were tightly taped to the sides of the pan with high grade zinc oxide tape 1 inch wide. This tape was better than cellulose acetate tape (Scotch tape), especially in making tight corners, and in the ease of removal.

Petri dishes of various sizes were very convenient for small samples. When these were used, the tops and the bottoms, respectively, were fitted in

pairs. Zinc oxide tape was used to cover the joint. Wire screen, surgical gauze, and tissue were placed over $CaCl_2$ crystals as in the pans.

Virus-infected leaves were removed from young, vigorous plants. With tobacco, the midribs were removed from large leaves and discarded. Tissues were clipped with shears into small pieces and spread on the surgical gauze in the pans or dishes.

After desiccation, the tissues were stored in bottles with screw caps. Caps were kept tightly in place to exclude moisture, or they were placed loosely on the bottles, and the bottles placed in tight cans containing $CaCl_2$ crystals. Storage was at $1°-2°$ C. unless noted otherwise.

Inoculations were made by wiping water extracts of the dried tissues on leaves dusted with carborundum powder.

All viruses were tested adequately at each assay to make certain that the surviving viruses were those under study, and not contaminating resistant viruses that were undetected in the original cultures.

The following viruses were studied:

Marmor cucumeris var. *vulgare* H.	Cucumber-mosaic virus
M. cucumeris var. *commolinae* H.	Southern celery-mosaic virus
M. anularium McK.[3]	Tobacco-ring-spot virus
M. cucumeris var. *upsilon* H.	Potato "Y"-mosaic virus
M. erodens H. (var. ?)	Tobacco-etch virus
M. medicaginis H.	Alfalfa-mosaic virus
M. terrestre var. *typicum* McK.[4]	Oat-apical-mosaic virus
M. terrestre var. *oculatum* McK.[4]	Oat-eyespot-mosaic virus
M. tritici var. *typicum* McK.[3]	Wheat-mosaic-rosette virus
M. tritici var. *fulvum* McK.[3]	Prairie-wheat-yellow-mosaic virus

These viruses become inactive in from a few days to a few weeks when the tissues are dried in the ordinary manner in the laboratory.

RESULTS

Since rapid drying reduces fermentation processes in leaf tissue, preliminary tests were conducted with desiccants at room temperature, and at higher temperatures with an isolate of cucumber-mosaic virus from squash and with the Southern celery-mosaic strain.

The results in table 1 show that some virus survived rapid desiccation of leaf tissues at the higher temperatures, but the level of survival seemed not sufficiently high to justify a full exploration of labile viruses by this method at this time.

The results in table 2 indicate that desiccation at temperatures just above freezing favors the survival of all but one of the labile viruses studied. No

[3] McKinney, H. H. Descriptions and revisions of several species of viruses in the genera Marmor, Fractilinia and Galla. Jour. Washington Acad. Sci. 34: 322–329. 1944.
[4] McKinney, H. H. Mosaics of winter oats induced by soil-borne viruses. Phytopath. 36: 359–369. 1946.

TABLE 1.—*Virus survival in leaf tissue desiccated at room temperature and above*

Virus	Host	Method of desiccating finely cut leaf tissue	Days dried	Test plant	Results[a]
Cucumber mosaic	Sweet corn	Dried in oven at 35° C. for 20 hr., stored over CaCl₂ at 23° C.	19	Tobacco	5/5
Do	do	do	39	do	2/5
Do	do	do	39	Corn	1/40
Do	do	do	58	Tobacco	2/2
Do	do	do	58	Corn	3/41
Do	do	Spread in lab. at 18.5° C.	9	do	0/45
Do	do	do	9	Tobacco	0/5
Do	do	In CaCl₂ desiccator at 18.5° C.	18	do	5/5
Do	do	do	42	do	2/2
Do	do	do	42	Corn	17/17
Do	Tobacco	Dried in oven at 53°–60° C. for 3¾ hr., tested immediately	.	Tobacco	0/5
Southern celery mosaic	Sweet corn	Dried in oven at 35° C. for 22 hr., tested immediately		Corn	1/32
Do	Cucumber	Dried in CaCl₂ desiccator in oven at 35° C. for 22 hr., tested immediately	. .	do	6/44
Do	Sweet corn	Dried in CaCl₂ desiccator in oven at 35° C. for 18 hr., stored at 1°–2° C.	27	do	2/112
Do	do	do	27	Cucumber	3/12
Do	do	CaCl₂ desiccator at 18.5° C.	24	do	1/13

[a] Numerators of fractions indicate the number of plants infected, and the denominators the number of plants inoculated.

tests were conducted at subfreezing temperatures, in oxygen-free atmosphere, or in vacuum to determine if further advantages may result.

DISCUSSION AND CONCLUSIONS

With the exception of the virus of oat eye-spot mosaic, the labile viruses tested survived desiccation for rather long periods. The oat-apical-mosaic virus showed slight activity after desiccation. However, as the oat-mosaic viruses are very difficult to transmit when the fresh extracts are used, the effect of desiccation is not apparent.

TABLE 2.—*Virus survival in finely cut leaf tissue desiccated over calcium chloride crystals at 1° to 2° C. Stored dry in tight bottles after desiccation at 1°–2° C.*

Virus	Host	Days dried	Test plant	Results[a]
Cucumber mosaic	Tobacco	125	Tobacco	5/5
Do	Sweet corn	669	do	5/5
Southern celery mosaic	do	613	do	5/5
Tobacco ring spot	Tobacco	393	do	5/5
Alfalfa mosaic	Cucumber	303	do	5/5
Potato "Y" mosaic	Tobacco	78	do	5/5
Do	do	420	do	6/10
Tobacco etch	Tobacco	301	do	10/10
Wheat mosaic rosette	Wheat	290	Wheat	10/38
Prairie wheat yellow mosaic	do	290	do	33/40
Oat apical mosaic	Oats	177	Oats	4/39
Oat eye-spot mosaic	do	177	do	0/41

[a] Numerators of fractions indicate the number of plants infected, and the denominators the number of plants inoculated.

Incidental tests have indicated that the host species is not a very important factor influencing survival of virus when desiccated. It appears that the major importance of the host lies in its ability to produce a large quantity of virus.

The results seem to justify the conclusion that, for the viruses studied, relatively rapid death in tissues dried in the ordinary ways at room temperature cannot be accounted for entirely on the basis of oxidation or desiccation. Desiccation may be a contributing factor, but it appears that other factors, such as fermentation processes, play a more direct rôle in bringing about this relatively rapid destruction of virus in tissues that are dried by the ordinary methods.

PLANT INDUSTRY STATION,
    BELTSVILLE, MARYLAND.

PHYTOPATHOLOGICAL NOTES

Cuticle Cracks in Tomato Fruits.—Cracks in the cuticle of mature-sized green tomatoes (*Lycopersicon esculentum* Mill.) were first noticed at the Tomato Disease Laboratory at Jacksonville, Texas, on July 12, 1937 (Fig. 1). These cracks spoiled the appearance of the fruits but they appeared too late in the marketing season to cause serious economic loss. However, in the week of June 25 to July 1, 1945, farmers lost the No. 1 grade price on many carloads of green-wrap tomatoes due to cuticle cracking. This abnormality was closely correlated with the weather at that time. Rains totalling 1.38 inches fell on June 22 and 23. On June 24 the temperature was as

Fig. 1. A. Tomato fruit with cuticle cracks that became deep enough to expose the epidermal cells to air so that they became black. B. Tomato with areas of blackened and sunken cuticle cracks.

high as 90° F. and small cracks, visible only by reflected light, developed abundantly in the cuticle of most of the large green tomatoes in most fields. The cuticle cracks occurred only in the dark green tops within 2 cm. of the pedicels. The cracks became more abundant in the next two days when temperatures were 90° to 92° F. They remained hyaline during the first day or two until they deepened enough to cause drying of the epidermal cells when they became black or brown (Fig. 1, A). Within 5 days, the areas with numerous cuticle cracks became sunken and black, making spots 3 to 12 mm. in diameter (Fig. 1, B).

Similar cuticle cracks were less abundant in the green and red fruits of tomatoes in November, 1945. Most of these cracks were discernible only with reflected light because they remained hyaline. They had formed in the period between Nov. 11 and 21 at temperatures of 45° to 80° F. with only 0.1 inch of rain on five of these days. The soil contained adequate water from the 1.96 inches of rain on Nov. 9 and 10. The epidermis was stripped from some of the affected fruits, mounted in water on microscope slides, and examined to determine the nature of the striations. The cuticle cracks ranged from 25 to 3000 μ long, 12 to 45 μ wide, and 100 to 300 μ apart

(Fig. 2).   The perpendicular fissures were about 24 µ long, 2 µ wide, and 3 to 6 µ apart in the longest crack illustrated (Fig. 2).   A few of the cracks were branched.

Most of the cuticle cracks were short arcs of circles that were concentric around the pedicel and probably resulted from the spherical stretching of the cuticle that was associated with increase in the polar diameter of the fruit (Fig. 2, C, S).   Some of these cracks apparently were stretched endwise soon after they formed (due to surface expansion that was associated with increase in the equatorial diameter), which made perpendicular fissures in some cracks (Fig. 2, F).
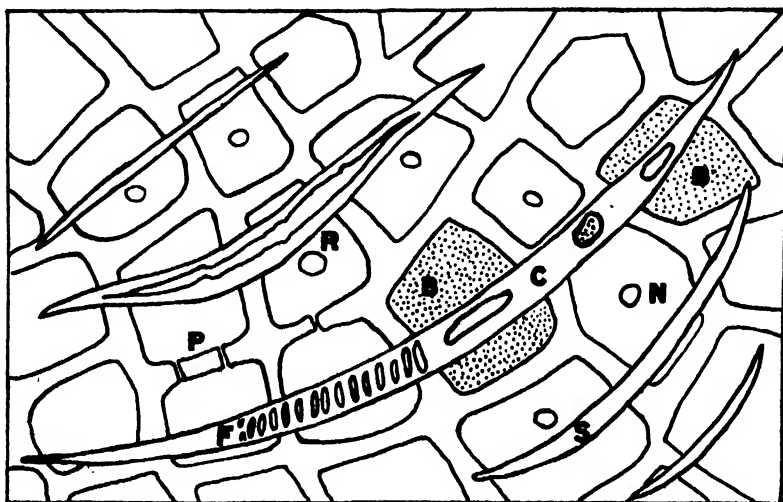
FIG. 2. Semidiagrammatic drawing of tomato-fruit epidermis with cuticle cracks above the epidermal cells. Shallow cuticle cracks (S) did not become deep enough to expose epidermal cells to air so they remained hyaline. Cuticle crack (C) deepened enough to expose two epidermal cells (B) to air so that they became black while three other underlying cells remained hyaline. Short fissures (F) formed almost perpendicular to the direction of the long cuticle crack that contained them. One cuticle crack (R) possibly ruptured first below the outer surface after which a ragged rift formed in the top. Nucleus (N). Plasmodesmata (P).

In 1946, abundant cuticle cracking was noticed first on June 24.   It was associated with rains totaling 5.21 inches that occurred on June 20 and 21, and an additional 2.34 inches of rain fell in 9 showers by July 2, but the temperature remained in the range of 68° to 86° F.   In recording yields on July 2 in a field of Rutgers tomatoes, 947 fruits were counted of which 18 per cent had cuticle cracking that was mild in about half of the fruits. Cuticle cracking was much more destructive in 1945 than in 1946, probably because hotter weather followed the rains in 1945.

The cuticle cracking described was in commercial varieties of green-wrap tomatoes with unripe fruits that had dark green stem-ends (due to UU-genes).   Less cuticle cracking occurred in tomatoes that had uniformly colored whitish-green unripe fruits (due to the uu-genes).   Four selections

of such tomatoes had only about one per cent of their fruits with cuticle cracking in 1946.   Of these, Selection No. 3 of the Southern Tomato Exchange Program had one fruit with cuticle cracks that were bordered by brown epidermal cells.   These cracks contained large air bubbles and small sand grains when the strip of tomato peel was mounted in water.   Three of the selections of un-gene tomatoes, including Crack-Proof variety, were resistant to both cuticle cracking and stem cracking.

Although they differ much in size and final symptoms, cuticle cracks probably have the same physiological causes as the deep subcuticular cracks that are commonly named stem cracks and growth cracks, and these two kinds of cracks often occurred in the same fruits.[1]   The stem cracks were 1 to 5 mm. deep and 1 to 6 cm. long, and some cracks crossed the blossom-end of the fruits, especially in Trip-L-Crop variety.   Rutgers and Marglobe tomatoes usually have radial stem cracks while Louisiana Red tomatoes usually have concentric stem cracks.   This indicates that these dissimilar varieties differ in their resistance to cracking when the peel is stretched by the swelling that is associated with increase in equatorial diameter versus increase in polar diameter.

Groth[2] noticed cuticle cracks in tomato skin and mentioned that the colorless cells around the cracks were unusual because they did not stain with haematoxylin.   Frazier[3] recorded a netting or russeting of Break O'Day tomatoes that may be related to cuticle cracking.   Iverson[4] said that stem cracking resulted from application of water to the fruits or soil.   Doolittle[5] illustrated common kinds of growth cracks.   Most stem cracking was due to uneven periods of swelling of the fruits, and control depended on maintaining practically uniform water supply and rate of growth (Young[6]).— P. A. YOUNG, Texas Agricultural Experiment Station, Jacksonville, Texas.

Rusty Spot of Peach.—In 1941 the writer called attention to a peculiar condition affecting peach fruits in Idaho, the symptoms of which suggested the name rusty spot.[1]   Subsequent observations indicate that the name is appropriate, particularly in the early stages of the disease (Fig. 1, C).   The pubesence of portions of the young fruits eventually sloughs off, leaving unsightly, bald patches of varying sizes (Fig. 1, C, D).   In comparison with normal-shaped pits those from severely affected rusty-spot fruits are smaller and thinner and the tips are curved toward the side of the severe rusty-spot area (Fig. 1, A, B).

[1] Young, P. A.   Cuticle cracking in green tomato fruits.   (Abstr.) Phytopath. **36**: 413.   1946.

[2] Groth, B. H. A.   Structure of tomato skins.   New Jersey Agr. Exp. Sta. Bul. 228. 1910.

[3] Frazier, W. A.   Types and severity of fruit cracking in tomato varieties.   Proc. Amer. Soc. Hort. Sci. **34**, 536.   1937.

[4] Iverson, V. E.   Fruit cracking of tomatoes.   Montana Agr. Exp. Sta. Bul., 362. 1938.

[5] Doolittle, S. P.   Tomato diseases.   U. S. Dept. Agr. Farmers Bul. 1934.   1943.

[6] Young, R. E.   Trellis tomatoes.   Massachusetts Agr. Exp. Sta. Bul. 419.   1944.

[1] Blodgett, Earle C.   Rusty spot of peach.   U. S. Dept. Agr., Pl. Dis. Rptr. **25**: 27–28. 1941.

FIG. 1. Rusty spot of Elberta peach naturally affected. A. Seeds of severely affected rusty-spot fruits showing the small, curved pits. B. Normal peach seeds. C. Young fruits showing the very early stages of rusty spot. D. Nearly mature fruits showing severely affected areas resulting in russeting, cracking, and malformation.

Although the exact nature of the cause of rusty spot is not yet determined, renewed interest is shown over this peculiar disease. In personal correspondence with E. L. Reeves in Washington, Gilbert Stout in California, and H. R. McLarty in British Columbia, the presence of rusty spot in these areas has been noted. In a few cases in California and Washington the disease has assumed serious proportions, especially on certain varieties.

In September, 1940, in the plots at Moscow, Idaho, 7 peach seedlings were bud-inoculated with 3 buds each of material from the orchard where the disease was first reported. No tree or leaf symptoms were noted until August, 1945, when it was observed that most of the fruit on 6 of the bud-inoculated trees were slightly to severely affected with rusty spot. One bud-inoculated tree bore healthy but no rusty-spot fruit.

Five of the bud-inoculated trees produced typically affected fruit; one tree bearing 21 rusty-spot and 3 healthy fruit, and another tree bearing 22 rusty-spot and 11 healthy fruit. Unfortunately, it was impossible to ascertain with certainty whether these 5 trees represented the under stock or the development of the scion. Fruit type was typical of the Elberta grown in the original orchard and the trees probably are from the scion shoots.

One of the bud-inoculated trees, however, which bore affected fruit was undoubtedly a seedling and represented the original under stock. The fruit was round and green while that from the other trees was elongated and more nearly mature. There is therefore indication that, in this case, the factor causing rusty spot was either transmitted or provided contamination from the scion buds. Adjacent trees and other trees in the plots, some checks and some inoculated with various bud-wood collections, showed no similarly affected fruit except in one case of an adjacent tree. This was a peach seedling which was inoculated in 1940 with buds from red-leaf chokecherry and bore only one rusty spot and several healthy peach fruits. Further observation substantiates that the rusty spots on the fruits are not due to infection by the ordinary powdery-mildew fungus.

Whether the factor is transmissible (as a virus), or perpetuated (as a genetic abnormality), or carried mechanically (as an organism), the results point out the danger of using bud wood from affected trees for propagation purposes. The occurrence of rusty spot on one tree not inoculated with affected material indicates the possibility of orchard spread.—EARLE C. BLODGETT, Formerly at Idaho Agricultural Experiment Station, Moscow, Idaho.

THE USE OF LEAF TISSUE IN GRAFT-TRANSMISSION OF PSOROSIS VIRUS[1]

JAMES M. WALLACE[2]

INTRODUCTION

Inoculation of citrus with psorosis viruses by means of small bark-patch grafts was reported recently.[3] Further investigations have shown that similar grafts, using leaf instead of bark tissue, give equally satisfactory transmission. This proves to be an advantage in that it speeds up inoculation procedure and eliminates the difficulty sometimes encountered in cutting bark patches from specimens from which the bark does not "slip" easily.

Sreenivasaya[4] reported transmission of the virus of the spike disease of sandal when rectangular pieces of leaf tissue from diseased plants were placed under bark flaps of healthy plants. Inasmuch as spike-disease virus is not transmissible by means of juice inoculations, there was probably actual union of the tissue in such "grafts."

Cochran and Rue[5] have reported that the virus of peach mosaic has been transmitted by placing diseased leaf tissue under the bark of healthy peach trees.[6] They observed that the patches were alive 75 days after grafting.

INOCULATION METHODS

In the inoculation of citrus with the psorosis virus by means of leaf-tissue grafts, the procedure is much the same as that previously described by Wallace for bark-patch inoculation. Rectangular pieces are cut from leaves of diseased trees and placed under bark flaps on healthy trees. The bark flap is folded back in position over the inserted leaf piece and held in place by a strip of adhesive rubber tape. It has been found that tissue from very young, newly formed leaves, as well as from fully mature leaves, can be used for transmission of the psorosis virus. The leaf piece need not fit exactly the exposed wood under the bark flap but should be small enough to be completely covered by the flap, so as to provide more opportunity for tissue union.

[3] Wallace, James M. Technique for hastening foliage symptoms of psorosis of citrus. Phytopath. 35: 535–541. 1945.

[4] Sreenivasaya, M. Contributions to the study of spike disease of sandal. Part XI. New methods of disease transmission and their significance. Indian Inst. Sci. Jour. 13 A (Pt. X): 113–117. 1930. (Original not seen by present writer; citation (68), p. 214 in: Smith, Kenneth M. Recent advances in the study of plant viruses. P. Blakiston's Son and Co., Inc., Philadelphia. 1934.)

[5] Cochran, L. C., and John L. Rue. Some host-tissue relationships of the peach mosaic virus. (Abstr.) Phytopath. 34: 934. 1944.

[6] In conversation, L. C. Cochran has informed the writer that, prior to the tests conducted by himself and Rue, mosaic infection resulted in peach trees inoculated by H. H. Thornberry by means of macerated leaf tissue placed under the bark; and that since peach mosaic is not transmissible by juice inoculations, the transmission obtained is assumed to have resulted from tissue union.

If the leaves used are fresh and clean, and if the bark of the inoculated tree "slips" easily, very few of the leaf sections fail to unite with the inoculated trees. Occasionally, however, the leaf sections dry or decay and transmission does not take place. In some instances the inoculated trees have not developed symptoms even though the leaf sections remained alive for a long time. To test the efficiency of this method of inoculation, 10 separate experiments were conducted with leaves of different ages and with different degrees of symptoms. The total number of trees included in the inoculation tests was 174, and of these, 160, or approximately 92 per cent, developed psorosis symptoms. This method is now being used, almost exclusively by the writer in psorosis studies, and many inoculation series have resulted in 100 per cent infection.

Seedling trees used in transmission studies were usually 5 to 7 mm. in diameter near the base of the main stem. Any tree that is of sufficient trunk diameter to permit peeling of a small bark flap can be inoculated in this manner, but it is somewhat easier to make this type of graft if the trees are 5 mm. or more in diameter.

Microscopic examination of sections of some of the leaf-tissue grafts showed that an increase in the amount of cut surface of the leaf piece provided more tissue capable of developing callus and thus gave more opportunity for tissue connections to form between the leaf pieces (the inoculum) and the trees in which they were placed. The procedure followed thereafter accordingly included an additional longitudinal cut through the center of the leaf piece, from points near the opposite ends. If leaf pieces included the midrib, a thin layer of tissue was shaved from the raised portion of the midrib.

A razor blade is suitable for making the cuts in preparing the bark flap, and for cutting the leaf pieces. A budding knife or scalpel is used to pry out the bark at the crosscut so as to permit the bark flap to be peeled downward.

ANATOMICAL STUDIES OF LEAF-TISSUE GRAFTS

The fact that the psorosis virus was transmitted from the leaf tissue to the seedling trees was evidence that a union of the tissues of the respective parts took place. Microscopic examination revealed the manner in which the tissues became united, and showed that union occurred only along the cut edges of the leaf pieces. Whenever entire, uninjured leaves were placed under the bark, there was no union.

Figure 1, A, shows a portion of a leaf-tissue graft sectioned and photographed 20 days after being placed under the bark. Callous tissue had formed at the cut edge of the leaf piece and had united with that formed by the cambium of the tree. It is evident that callous tissue developed from the direction both of the bark and of the wood. Where the cuticle and epidermis of the inserted leaf piece were not broken, there was no tissue union. When sections were mounted in water, the leaf portion separated

FIG. 1. A. Section of leaf-tissue graft 20 days after tissue was placed under bark flap. Note fusion of callous tissue at cut edge of leaf patch, and lack of connection where leaf epidermis was unbroken. ×72. B. Enlarged view of same section. ×144. C. Section through leaf-tissue graft in which midrib of leaf was included. Callus union resulted where the surface of the midrib was removed. ×72. D. Leaf-tissue graft in which additional cut was made through leaf piece. One month after grafting, callus had grown completely through the opening, and new xylem was again being produced on the bark side. ×72.

and floated free except at the attached edge. Figure 1, B, shows an enlarged view of the point of union of the respective parts.

Figure 1, C, shows a section through a leaf-tissue graft in which the midrib of the leaf was included in the leaf piece. A thin layer of tissue had been shaved from the under surface of the midrib. The unbroken surface of the leaf piece was placed adjacent to the xylem, with the bark flap in contact with the cut surface of the midrib. Fusion of tissues took place where the surface of the leaf piece was injured, but there was no tissue union on the opposite side, where the cuticle and epidermis were unbroken.

When a longitudinal cut was made through the leaf piece, callus formed by the seedling tree filled the opening and united with the cut edges of the leaf piece, as shown in figure 1, D.

Examination of some of the leaf pieces 30 months after they were placed under the bark showed them to be living and to have a normal green color, although they were then buried in 3 to 4 mm. of wood.

DISCUSSION AND SUMMARY

Inoculation by means of small, rectangular pieces of leaf tissue from psorosis-affected citrus trees, placed under bark flaps of healthy citrus trees, resulted usually in infection of 90 to 100 per cent of the inoculated trees.

Microscopic examination showed that callous tissue developed along the cut edges of the leaf piece and united with callus formed by cambial tissues of the inoculated tree. Fusion of tissues was rapid. The appearance of psorosis symptoms on the inoculated seedlings within 2 to 4 weeks, depending upon the rate of growth of new shoots, showed that the psorosis virus moved into them from the leaf pieces (the inoculum) within a few days after the grafts were made.

Numerous leaf pieces examined 30 months after being placed under the bark were still living and were of normal green color even though they were then buried in 3 to 4 mm. of wood.

Citrus may be especially adapted for the type of leaf-tissue graft described in this paper, but the successful transmission of viruses of sandal and peach by similar grafts suggests that this method may be applicable to a wide range of plants. It has not yet been determined whether virus transmission can be accomplished with this technique between plants that are not compatible when budded or grafted by the usual methods. If such should prove to be the case, studies of the host range of virus disease will be facilitated.

With plants in which this method of grafting can be used for virus transmission, inoculation procedure is simplified and inoculations can be made rapidly. The method may also prove of value in studies of rate of movement and distribution of viruses in plant tissues.

UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION,
RIVERSIDE, CALIFORNIA.

A CHEMICAL STUDY OF THE MYCELIUM AND SCLEROTIA OF PHYMATOTRICHUM OMNIVORUM[1]

DAVID R. ERGLE AND LESTER M. BLANK

Since 1888 (11) *Phymatotrichum omnivorum* (Shear) Duggar, a soil-inhabiting fungus, has been the subject of a vast amount of research. Much of the earlier work has been summarized by Rea (12) and Streets (14). Information on the chemical composition of the fungus appears to be limited to that by King (8), who found the conidia to contain 1.73 per cent organic nitrogen, and by Rogers (13), who reported, on the basis of microchemical tests, the presence of cellulose, protein, starch, and suberin in naturally occurring sclerotia recovered from field soils.

In the study reported in this paper *Phymatotrichum omnivorum* was cultured in three different soil types containing sorghum seed as the primary source of food, the variations in certain constituents of sclerotia during growth were determined, and these are considered in the light of their possible physiological significance. A similar study was made of mycelium grown in a nutrient solution. In the field this form of the organism, which brings about root infection of susceptible hosts, may be seen as a delicate weft of slender filaments and heavier strands, tan to buff colored, encompassing the roots.

Past investigations of the fungus relative to its occurrence and activity in certain soil areas of the Southwest have justified two conclusions that were considered in planning the work described in this study. (a) Sclerotia, which are compact tuber-like swellings of large mycelial strands, constitute one of the most important means by which the fungus persists in the soil. They are known (16) to have remained viable in the soil for at least 8 years, and have been found in large quantities to depths of 8 feet in Houston Black clay soil (13). (b) Soil type, composition, and reaction appear to be important factors determining the ability of the fungus to maintain itself and the resulting severity of the disease (5, 6, 18). The extent of infestation and severity of damage by the fungus is favored in heavy soils of neutral to moderately alkaline reaction and inhibited in the acid soils.

CULTURE AND PREPARATION OF PHYMATOTRICHUM OMNIVORUM FOR CHEMICAL ANALYSIS

Because of the importance attached to soil type in determining the distribution and activity of the fungus, three soils associated with varying degrees of natural infestation were used as substrates for obtaining suitable quantities of sclerotia for chemical study. They were as follows: (a) Houston Black clay, high-lime phase (14.44 per cent CaO), pH 8.1, obtained from

[1] Published with the approval of the Director of the Texas Agricultural Experiment Station as Technical Paper No. 969.

a field highly infested with *Phymatotrichum omnivorum* near Temple, Texas.
(b) Houston Black clay, low-lime phase (1.55 per cent CaO), pH 7.9, obtained near Moulton, Texas, from a field in which the degree of infestation appears to be less than in (a). (c) Wilson clay, noncalcareous, pH 4.4, obtained near Greenville, Texas, in a field in which there was little infestation.

Sclerotia were obtained by the soil-culture method of Dunlap (2) whereby the fungus was cultured in 100 g. soil plus 35 ml. tap water, and 5 g. sorghum seed spread over the surface. The mixture, contained in a 250-ml. Erlenmeyer flask, was sterilized by autoclaving. Inoculation was by means of a small disc of nutrient agar containing an established culture of the fungus isolated from a naturally infected cotton plant. Using the soils described above, three series of 65 flasks each were thus prepared and incubated at 28° to 30° C. Seventeen days after inoculation, the sclerotia formed by the fungus were separated from the soil in 20 flasks of each soil type by washing with water and screening. After removal of the surface water by blotting, the sclerotia were weighed as a composite sample for each soil type, dried in a forced draft oven at 75° C., and then reweighed to obtain a dry weight of the sclerotia. Preparation for immediate analysis consisted of grinding in a small Wiley mill to pass an 80-mesh screen and redrying for weighing of analytical samples. Similar harvests of sclerotia from 15 flasks in each series were made 33, 49, and 65 days after inoculation, representing successive stages in sclerotial growth and development. Preliminary experiments had demonstrated that the peak in sclerotial growth was reached in approximately 65 days of incubation under the conditions described.

While sclerotia grew best in soil cultures, the mycelium could not be separated in quantity free of soil particles. So mycelium for chemical analysis was grown on 50 ml. of nutrient (15) solution in 250-ml. Erlenmeyer flasks. Inoculation and incubation were like those for the soil cultures. When fungal growth was near its maximum after 16 days, the mats from 15 flasks were harvested, washed free of nutrient solution, dried for 3 hours, and prepared for analysis as described for sclerotia. A second harvest was made after 22 days from 15 more flasks. During these last 7 days autolysis began. The mean dry weight of fungal mat per flask at the first harvest was 867 mg. and at the second harvest was 833 mg.

ANALYTICAL PROCEDURE

Ash, crude fat, and organic nitrogen (nitrates were absent) were determined by A.O.A.C. official methods (1). Crude protein was calculated by multiplying nitrogen by the usual conversion factor, 6.25.

The sugars were Soxhlet-extracted with 80 per cent alcohol and determined by the method of Wildman and Hansen (19). Nonreducing sugars were determined after hydrolysis with 4 per cent hydrochloric acid (by weight) for 1.25 hours at 15 lb. pressure. The methods of analysis as out-

lined by Loomis and Shull (10) formed the basis for the following determinations in the order given: (a) Glucosans and pectin were determined on separate aliquots of a hot-water extract of the residue from the extraction of sugars. Pectin was precipitated as the calcium salt according to the procedure of Emmet and Carré (4). (b) The residue from (a) was hydrolyzed with 2.5 per cent hydrochloric acid for 1 hour at 15 lb. pressure and separate aliquots of the hydrolysate used for the determination of hemicellulose and pentosans. (c) The washed and dried residue from (b) was treated with 72 per cent sulphuric acid for 24 hours at 8° C. followed by a 3 per cent sulphuric acid hydrolysis at 100° C. for 2 hours. The reducing power of the hydrolysate, calculated as anhydrous dextrose, was used as an index of the cellulose content. Inasmuch as tests for lignified tissue in mycelium and sclerotia were negative whereas the presence of suberin in sclerotia was confirmed (13), the residue from the cellulose determination, after correcting for its ash content, was reported as suberin.

The presence of suberin was confirmed by the formation of a yellow to brown color when sections of fresh tissue were treated with chloro-zinc iodide (7). Glycogen was identified by the opalescence of the glucosan extract, by a reddish brown color with iodine that was intensified by sodium chloride and by the conversion to glucose upon acid hydrolysis (9).

<div align="center">RESULTS</div>

It is believed that sclerotia formation was limited for the most part to the initial 17-day incubation period and that the succeeding periods represent their continued growth and development. Such an assumption is supported by two types of experimental evidence. First, sclerotia formed in the 17-day-old cultures were invariably lighter in color and smaller than those in older cultures. Second, the nutrient reserves of sclerotia increased consistently with length of incubation period, thus indicating that they were older physiologically.

<div align="center">*Sclerotia*</div>

Analytical data on the changes in chemical composition of sclerotia with age of culture in the three soils are recorded in table 1 as percentage of fresh weight. The production and cumulated growth of sclerotia in the three soils over the 65-day incubation period is shown in figure 1, A. Each point on the curves is the mean dry weight of sclerotia per culture computed from not less than 15 replicates. As shown by these curves the efficiency of the fungus in sclerotia production was in the following order: Houston Black clay—high lime, Houston Black clay—low lime, and Wilson clay. These results confirm similar observations reported by Dunlap (2).

As illustrated in figure 1, B, growth was accompanied by a marked decrease and ultimate disappearance of reducing sugars from the sclerotia. The concentration of reducing sugars in sclerotia varied inversely with rate of growth in the three soils whereas the rate at which these sugars disappeared tended to be directly related to growth. This is interpreted as

indicating an effect of soil type on the ability of sclerotia to utilize the sugars made available by enzymatic hydrolysis of the sorghum seed. Apparently the sclerotia in the Wilson soil were getting adequate amounts of sugars, as indicated by a higher content, but were unable to convert them into the various products of synthesis as fast as sclerotia growing in the two Houston soils.

TABLE 1.—*Chemical composition of Phymatotrichum sclerotia at four stages of growth in three different soils. Results given as percentage of fresh weight*

Soil type and constituents	Age of culture—days			
	17	33	49	65
Houston Black clay (High lime)				
Moisture	74.24	60.76	57.53	60.34
Ash	1.80	1.48	1.36	1.30
Fat	0.55	0.80	0.89	0.88
Protein	4.70	6.13	7.73	7.84
Reducing sugars	0.26	0.04	0.00	0.00
Nonreducing sugars		0.56	0.48	0.68
Glucosans	1.26	3.86	4.37	6.07
Hemicellulose	8.37	13.34	14.64	14.84
Pentosans		0.16	0.17	0.15
Pectin		0.80	1.15	0.83
Cellulose	0.28	0.33	0.39	0.39
Suberin	0.33	0.82	0.73	0.54
Houston Black clay (Low lime)				
Moisture	75.20	62.24	59.73	57.03
Ash	1.67	2.07	1.79	1.38
Fat	0.62	0.89	0.96	1.13
Protein	4.26	5.85	6.79	7.55
Reducing sugars	0.31	0.16	0.03	0.00
Nonreducing sugars		0.63	0.63	0.84
Glucosans	1.63	3.11	4.85	6.28
Hemicellulose	8.57	14.20	14.24	16.16
Pentosans		0.11	0.15	0.16
Pectin		0.65	1.24	0.67
Cellulose	0.26	0.31	0.46	0.36
Suberin	0.36	0.53	0.60	0.54
Wilson clay (Noncalcareous)				
Moisture	75.76	62.46	60.45	57.34
Ash	2.47	2.80	2.88	1.74
Fat	0.48	0.74	1.05	1.20
Protein	4.53	5.77	6.27	7.52
Reducing sugars	0.44	0.33	0.06	0.00
Nonreducing sugars		0.53	1.07	0.99
Glucosans	0.68	3.05	3.35	4.00
Hemicellulose	7.47	15.73	12.92	18.80
Pentosans		0.21	0.15	0.20
Pectin		0.56	0.66	0.80
Cellulose	0.26	0.32	0.34	0.41
Suberin	0.40	0.53	0.72	0.66

Concurrently with growth and development, the sclerotia, irrespective of soil type, increased consistently in concentrations of fat, protein, glucosan, and hemicellulose. The capacity of sclerotia to accumulate protein and glucosans (Fig. 1, C and D) was apparently influenced moderately by the kind of soil used as culture medium.

The concentrations of nonreducing sugars, pentosans, pectin, cellulose, and suberin in sclerotia remained relatively small throughout the growth

period and were not affected by the nature of the substrate. Excepting pentosans these constituents did, however, tend to increase in concentrations as the sclerotia approached maturity.

The moisture content of sclerotia decreased with age without any differences attributable to soil type. The ash content varied inconsistently with respect to growth but was always present in higher concentrations in sclerotia from the Wilson soil.
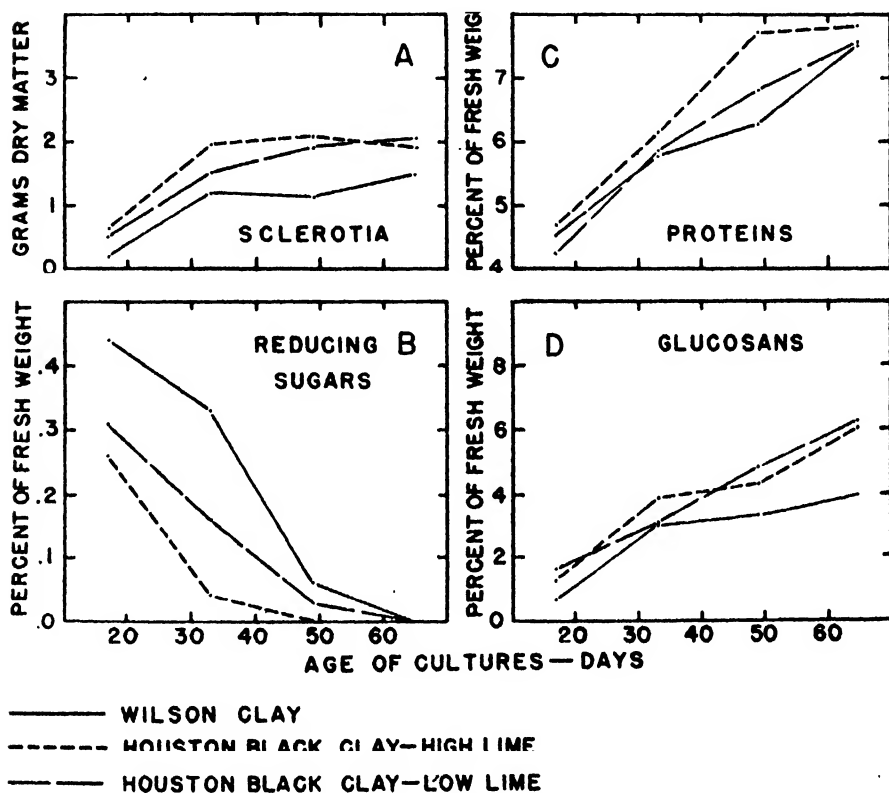
FIG. 1. Graphs showing the influence of soil type on (A) growth of sclerotia; (B) rate of disappearance of reducing sugars; and accumulation during growth of (C) proteins and (D) glucosans.

Mycelium

The data comparing the chemical composition of mycelium and sclerotia at two stages of comparable physiological age are recorded in table 2 as percentage of dry weight. The use of the moisture-free basis was advisable due to the absorption of water by the mycelial mats during washing, thereby precluding a representative fresh weight.

As grown, the mycelium reached a maximum weight in 16 days and, as a result of some autolysis, was declining by the 22nd day. This is about half of the growth period of sclerotia in the soil culture. The mycelium at

all dates of measurement was higher than the sclerotia in concentrations of ash, fat, protein, reducing sugars, pectin, cellulose, and suberin. Sclerotia, on the other hand, were definitely higher in concentrations of nonreducing sugars, glucosans, and hemicellulose. At the peak of growth, mycelium and sclerotia had accumulated total carbohydrates (sugars + glucosans + hemicellulose) in concentrations of 23.16 and 45.92 per cent of their dry weights, respectively. With reference to the types of carbohydrates accumulated, the mycelium was richer in sugars whereas sclerotia were more abundantly supplied with polysaccharides. This is a more or less expected

TABLE 2.—*Comparison of chemical composition of mycelium and sclerotia of Phymatotrichum at two stages of growth. Results given as percentage of dry weight*

	Mycelium			Sclerotia		
	Age of culture—days		Means	Age of culture—days		Means
Constituents	16	22		49	65	
Ash	6.12	6.35	6.24	3.20	3.29	3.25
Fat	4.03	4.95	4.49	2.10	2.23	2.17
Protein	23.75	24.13	23.94	18.19	19.77	18.98
Reducing sugars	4.74	4.34	4.54	0.00	0.00	0.00
Nonreducing sugars	0.00	0.00	0.00	1.14	1.72	1.43
Glucosans	1.48	0.93	1.21	10.30	15.30	12.80
Hemicellulose	16.94	20.37	18.66	34.48	37.41	35.95
Pentosans	0.45	0.47	0.46	0.41	0.38	0.40
Pectin	6.36	3.10	4.73	2.71	2.09	2.40
Cellulose	2.46	3.17	2.82	0.91	0.99	0.95
Suberin	2.69	2.86	2.78	1.73	1.36	1.55

result since the mycelium may be considered to be more active physiologically and of more temporary duration than sclerotia, and as such might be expected to accumulate carbohydrates in more labile forms.

Other Constituents

Some information was obtained relative to the chemical character of the carbohydrates involved in the synthetic processes of the two fungal forms. A nonreducing sugar, hydrolyzed to glucose with difficulty, was present in sclerotia but not in the mycelium. Although the identity of this compound was not definitely established, its behavior on acid hydrolysis is suggestive of trehalose, a disaccharide of fairly wide distribution among fungi. Upon strong acid hydrolysis trehalose yields two molecules of glucose. Neither starch nor sucrose was present in either of the two stages of the fungus. Thus the presence of starch in sclerotia, as reported by Rogers (13), was not confirmed. Hot water extracts of both mycelium and sclerotia contained a polysaccharide identified as glycogen that was included in the glucosan determination. Glycogen is a common reserve carbohydrate in other fungi such as mushrooms and yeast. The hemicellulose fraction in both types of tissue was low in pentosans and upon acid hydrolysis yielded glucose as the principal sugar. There was some evidence that glycogen

was present also in the hemicellulose fraction. These findings indicate that the synthesis of polysaccharides in this fungus was limited for the most part to the utilization of glucose as polymer units.

DISCUSSION

The results of this study aid materially in explaining from a nutritional standpoint why the sclerotial form of *Phymatotrichum omnivorum* is able to play an important rôle in the perpetuation of the organism in field soils. The demonstrated ability of sclerotia during growth to accumulate carbohydrates, fat, and protein in relatively high concentrations suggests that their physiological function is primarily that of a storage organ. Such a concept accords with the known behavior of this form of the fungus as it occurs naturally in soils infested with *Phymatotrichum omnivorum*. In such an environment, sclerotia may remain dormant for long periods but dormancy may be broken by the advent of favorable moisture and temperature. The breaking of dormancy results in the formation of young hyphae which are capable of attacking the roots of susceptible hosts. It is obvious that the initial phase of hyphal production and expansion is at the expense of stored reserves, and that the amount of growth, independent of an auxiliary source of organic nutrients, is conditioned by the amount of stored nutrients. Therefore, the fungus with an abundant supply of food stored in sclerotia could be expected to survive for a time in soils kept free of susceptible plants.

The data comparing the chemical composition of the two fungal forms indicate that fat, protein, and carbohydrates can be expected to accumulate during growth in mycelium as well as in sclerotia. This similarity is not surprising in view of the fact that sclerotia are formed from mycelial strands. However, in view of the lower percentage of carbohydrates in the mycelium, it seems likely that the independent existence of the fungus in this stage would encounter a critical shortage of carbohydrates sooner than the sclerotial stage. Thus it is apparent that the mycelium is not so well adapted as sclerotia to prolonged survival under critical conditions.

The occurrence of suberin in mycelium and sclerotia may have a substantial significance from the standpoint of moisture retention. It has been shown elsewhere (3) that *Phymatotrichum omnivorum* is capable of making considerable growth from moist substrates out into soils of moisture content within the wilting range.

SUMMARY

Phymatotrichum omnivorum, a soil-inhabiting fungus, was cultured in (a) Houston Black clay, high-lime phase, (b) Houston Black clay, low-lime phase, and (c) Wilson clay soil, and the chemical composition of the sclerotia was determined at four stages of growth. Growth of sclerotia was accompanied by a disappearance of reducing sugars at a rate commensurate with growth and an accumulation of fat, protein, glucosans, and hemicellu-

lose. Corresponding with a higher rate of growth, glucosans and protein tended to accumulate in higher concentrations in sclerotia produced in the two Houston soils than in the Wilson soil. Nonreducing sugars, pectin, pentosans, cellulose, and suberin were present in sclerotia in relatively small amounts which, with the exception of pentosans, tended to increase with the approach of maturity but were not affected by soil type.

The mycelium of *Phymatotrichum omnivorum*, cultured in a suitable nutrient solution, was found to contain fat, protein, and reducing sugars in higher concentrations than sclerotia but manifested a substantially lower capacity than the latter for storing total carbohydrates (sugars + glucosans + hemicellulose). The sclerotial stage was richer in nonreducing sugars, glucosans, and hemicellulose. Ash, pectin, pentosans, cellulose, and suberin were also present in mycelium in larger concentrations than in sclerotia.

Starch and sucrose were absent in both fungal forms. A disaccharide, believed to be trehalose, was present in sclerotia but absent in the mycelium. Glycogen was identified in both forms of the fungus. Excepting the occurrence of small amounts of pentosans, the polysaccharides in both stages of the fungus appeared to be chiefly polymers of glucose.

Bureau of Plant Industry, Soils, and
　　Agricultural Engineering
　　　　and
Texas Agricultural Experiment Station,
　　College Station, Texas.

LITERATURE CITED

1. Assoc. Offic. Agr. Chem. Official and tentative methods of analysis. 4th ed. 1935.
2. Dunlap, A. A. A convenient soil-culture method for obtaining sclerotia of the cotton root rot fungus. Amer. Jour. Bot. 28: 945–947. 1941.
3. Eaton, F. M., E. W. Lyle, and D. R. Ergle. Relations between carbohydrate accumulation and resistance of cotton plants to Phymatotrichum root rot in dry summers. In press.
4. Emmet, A. M., and M. H. Carré. A modification of the calcium pectate method for the estimation of pectin. Biochem. Jour. 20: 6–12. 1926.
5. Fraps, G. S. Relation of the occurrence of cotton root rot to the chemical composition of soils. Texas Agr. Exp. Sta. Bull. 522. 1936.
6. Gilbert, W. W. Cotton diseases and their control. U. S. Dept. Agr. Farmers' Bull. 1187: 27–31. 1921.
7. Johansen, Donald Alexander. Plant Microtechnique. 1st ed. 523 pp. 1940.
8. King, C. J. Habits of the cotton rootrot fungus. Jour. Agr. Res. [U.S.] 26: 405–418. 1923.
9. Koch, Frederick C. Practical methods in Biochemistry. 1934.
10. Loomis, Walter E., and Charles A. Shull. Methods in Plant Physiology. 1st ed. 472 pp. 1937.
11. Pammel, L. H. Root rot of cotton or "cotton blight." Texas Agr. Exp. Sta. Bull. 4. 1888.
12. Rea, H. E. The control of cotton root-rot in the Blackland region of Texas. Texas Agr. Exp. Sta. Bull. 573. 1939.
13. Rogers, C. H. Cotton root rot studies with special reference to sclerotia, cover crops, rotation, tillage, seeding rates, soil fungicides, and effects on seed quality. Texas Agr. Exp. Sta. Bull. 614. 1942.
14. Streets, R. B. Phymatotrichum (cotton or Texas) root rot in Arizona. Ariz. Agr. Exp. Sta. Tech. Bull. 71. 1937.

15. TALLEY, PAUL J., and LESTER M. BLANK.   A critical study of the nutritional require-
    ments of *Phymatotrichum omnivorum*.   Plant Physiol. 16: 1–18.   1941.
16. TAUBENHAUS, J. J.   Laboratory studies on longevity of sclerotia.   Texas Agr. Exp.
    Sta. Ann. Rept. 49.   1936.
17. ——————, W. N. EZEKIEL, and J. F. FUDGE.   Relation of soil acidity to cotton
    root rot.   Texas Agr. Exp. Sta. Bull. 545.   1937.
18. ——————, ——————, and D. T. KILLOUGH.   Relation of cotton root rot and
    Fusarium wilt to the acidity and alkalinity of the soil.   Texas Agr. Exp. Sta.
    Bull. 389.   1928.
19. WILDMAN, SAM G., and ELMER HANSEN.   A semi-micro method for the determination
    of reducing sugars.   Plant Physiol. 15: 719–725.   1940.

INFLUENCE OF CERTAIN ENVIRONMENTAL CONDITIONS ON CHLOROTIC STREAK OF SUGAR CANE

E . V . A B B O T T [1]

Wherever chlorotic streak, a virus disease of sugar cane, has been studied it has been noted that incidence and severity of the disease may be influenced by the conditions under which the sugar-cane plant is growing. It has been observed to be particularly severe where drainage is poor, and there have been indications that its severity is enhanced by nutritional deficiencies. However, there has been little experimental evidence to support the field observations. In this paper the results of experiments on the effect of soil moisture and texture and of certain fertilizers on the disease are reported.

SOIL MOISTURE

The greater prevalence of chlorotic streak in low, poorly drained areas as compared with well drained ones has been observed in Queensland (3, 4, 5), Hawaii (9), and Louisiana (1). One instance of the association of the disease with high soil moisture in Louisiana, apparently independently of other soil differences, may be cited as a typical example.

In June, 1940, infection counts were made in a commercial field of the variety C.P. 29/320 plant cane growing on "mixed" soil[2] in Terrebonne Parish, the rear portion of which was low and poorly drained in comparison with the front part. The area of poor drainage lay roughly at right angles to the direction of the rows, so that it was possible to make observations in poorly and well-drained portions of individual rows. The percentage of diseased plants in the low areas varied from 2 to 4 times that in the well-drained portions of the same rows, and in every row this unequal distribution of diseased plants occurred, with a rather sharp line of demarcation corresponding with the differences in drainage. This could scarcely be explained on the basis of unequal distribution of infected seed cuttings in different parts of the rows, since both the front and rear portions had been planted from the same wagon. Evidently, some effect of poor drainage had either resulted in a greater development of leaf symptoms, or in less recovery from the disease during germination of the buds and subsequent growth of the plants.

Experimental evidence has been obtained to support this and other similar field observations. In the fall of 1940, composite samples of chlorotic-

streak-infected stalks of 2 varieties, C.P. 807 and C.P. 29/103, were divided into 2 lots each, 1 for planting in a poorly drained end of a field of mixed soil and the other in a well-drained portion of the same field.   There were 3 replications of 1/100-acre plots of each variety in each area, and in each plot 300 sound buds were planted.   In 1941, when stands were established and chlorotic streak symptoms had appeared in the young plants, the percentages of diseased plants were determined.   The number of plants of C.P. 807 with chlorotic streak symptoms was 54 per cent greater in the poorly drained than in the well-drained area, and in the C.P. 29/103 it was 78 per cent greater.

Since field observations had indicated that secondary spread of the disease was greater in areas of poor drainage, experiments were conducted to obtain more definite evidence.

In October, 1940, mature cuttings from apparently healthy plants of the variety C.P. 29/320 were treated with hot water at 52° C. for 20 minutes, which eliminates the disease from infected stalks, and divided into 2 lots for planting, respectively, in poorly and in well-drained portions of a field of "mixed" soil near Raceland, Louisiana.   The field was 580 feet long.   In the front 200 feet drainage was good, whereas in the rear 200 feet it was such that the soil remained wet for long periods following a rain, and water often stood for several days between the rows of cane, which in Louisiana are elevated about 12 to 18 inches.   Frequent soil-moisture determinations showed that throughout the season the moisture content was consistently higher in the poorly drained area.

Four replications of 3-row plots, 1/100 acre in size, were planted in each of the 2 areas, using 300 sound buds per plot.   Each plot was buffered by a row of chlorotic-streak-diseased cane on the sides, and by a similar 6-foot strip on each end.   Thus, exposure to secondary spread of the disease by aerial insects should have been uniform in both areas.

In June, 1941, counts of primary shoots were made in all of the plots. At monthly intervals thereafter during the growing season as plant cane, and as first ratoon in 1942, the stools developing symptoms of chlorotic streak were marked with permanent wooden stakes.   At the end of the ratoon year the percentage of infected stools was determined.

In October, 1941, a similar experiment was planted in a field in the same area and in which drainage conditions were similar.   Eight varieties were included, with 9 replications of single-row plots 25 feet long in each of the areas of good and poor drainage.   The seed cuttings were treated with hot water, the plots were buffered with chlorotic-streak-diseased cane, and notes were taken as described for the previous experiment.   This experiment was continued through the crop seasons of 1942 and 1943.   The results of the two experiments are in table 1.

Infection in the areas of poor drainage was significantly higher at the 5 per cent level for 7 of the 8 varieties included in the tests.   Spread of the disease was greater in the area of poor drainage in the resistant varieties,

C.P. 33/372 and C.P. 34/21, as well as in the susceptible ones, although for the former variety the difference was not significant.

The influence of soil moisture on the development of chlorotic streak was also studied in the greenhouse, where variables other than moisture could be better controlled.

Greenhouse benches, $72 \times 38 \times 8\frac{1}{2}$ inches, were filled with fresh field soil, 2 with Sharkey silty clay and 2 with Yazoo very fine sandy loam. Composite samples of single-bud cuttings from chlorotic-streak-infected stalks of C.P. 29/320 were prepared by placing alternate buds successively in 4 lots as they were cut. After incubation at 35° C. for 36 hours, which caused the buds to swell, 50 were planted in each of the benches in groups of 10 on December 11, 1941. Soil moisture was kept at or near optimum in all of the benches

TABLE 1.—*Effect of drainage on secondary spread of chlorotic streak in 8 varieties of sugar cane*

Varieties used in each experiment	Plants infected with chlorotic streak				
	Good drainage	Poor drainage	Difference	Diff. Req'd. for	
				P. = 0.05	P. = 0.01
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Experiment 1					
C.P. 29/320	16.4	29.8	13.4	2.7	9.0
Experiment 2					
C.P. 29/320	19.9	37.5	17.6	8.2	16.7
C.P. 32/206	42.8	55.4	12.6	9.1	18.7
C.P. 33/372	6.5	7.8	1.3	2.3	4.8
C.P. 34/21	4.1	7.4	3.3	2.2	4.6
C.P. 34/86	24.5	37.7	13.2	8.9	18.3
C.P. 34/92	22.5	30.9	8.4	4.9	10.2
C.P. 34/120	30.2	36.2	6.0	3.3	6.9
C.P. 34/139	35.6	58.3	22.7	8.0	16.4

until December 31, by which time the plants had become established. One bench of each soil was then flooded, and watered daily thereafter sufficiently to maintain the moisture level at within an inch of the surface of the soil. The soil in the other 2 benches was maintained at apparently optimum moisture content throughout the experiment.

When the experiment was terminated on March 9, 1942, 29 per cent of the plants in the sandy soil with high moisture had developed chlorotic streak symptoms, compared with 20 per cent in that with normal moisture; and in the heavy soil, 33 per cent developed symptoms with high moisture compared with 17 per cent with normal moisture. The differences were significant for both soils, confirming field evidence that high soil moisture is an important factor in favoring the development of chlorotic streak.

The foregoing experiments provide definite evidence not only of greater incidence of the disease among plants arising from infected seed when planted in poorly drained as compared with well-drained soils, but also of more rapid secondary spread, resulting apparently, from the greater susceptibility of plants growing under such conditions. It is possible that, as

suggested by Bell (4), the insect vectors of the disease are more active in the poorly drained areas. This is improbable, in the case of the one known insect vector *Draeculacephala portola* (2), which does not appear to be so limited in its movements as to confine itself to such relatively small areas in a field, but it is possible that the disease may be transmitted by some unknown soil-inhabiting vector that is more active in poorly drained areas. However, there are no data to support this, and the writer's efforts to transmit the disease through the soil have been unsuccessful. No infection of healthy plants occurred when these were grown for 9 to 10 months in alternate position with chlorotic-streak-infected ones in greenhouse benches of nonsteamed clay soil, the moisture content of which was maintained at a high level; nor when healthy plants were grown for 2 years in the greenhouse in cans of nonsteamed soil that had been collected from around the roots of diseased plants in the field.

In the field, extreme deficiency of soil moisture emphasizes the leaf scorching and streaking of diseased plants, so that during a drought the individual infected plants or areas of high infection stand out in sharp contrast to adjacent healthy ones. However, no evidence has been obtained to indicate that moisture deficiency results in higher incidence of the disease. On the contrary, in the field studies of secondary spread, more new cases appeared among originally healthy plants during the rainy summer months than during dry periods. It is realized, of course, that factors other than soil moisture, such as age and rate of growth of the plants, and seasonal activity of insect vectors, may be involved in the more rapid spread in summer.

SOIL TEXTURE

In Louisiana, poor drainage is associated more commonly with silty clay and clay soils, such as those of the Sharkey series (the so-called "black" lands), than with the lighter-textured sandy soils, such as those of the Yazoo series. The incidence of chlorotic streak has generally been found to be greater in sugar cane growing on the former as compared with the latter soils, and while it seemed probable that this was primarily because of the generally poorer drainage of the heavy soils, there remained the possibility that soil differences other than drainage might also have an influence on the disease. In the minds of many growers, at least, prevalence of the disease has come to be associated with the heavier soil types.

In order to determine whether soils of different texture held at or near their optimum moisture content would have any differential effect on the development of chlorotic streak, experiments were conducted in the greenhouse with sandy loam, and clay loam soil types and with muck soil.

Four composite samples of 150 single-bud cuttings from chlorotic-streak-infected stalks of the variety C.P. 29/320 were prepared as described previously for the greenhouse experiment. They were planted in 4-inch clay pots of soil, 2 lots of each in Yazoo very fine sandy loam and 2 in Sharkey.

silty clay loam. One lot of each soil was germinated at 35°–36° C. and the other at 22°–23° C. As the shoots emerged from the soil, the pots were removed to benches in the greenhouse where the minimum air temperature was maintained at 24° C. The date of germination and of the first appearance of chlorotic streak symptoms was recorded for each plant. The experiment was begun in October, 1940, and terminated in August, 1941.

The two germination temperatures were used to determine whether greater recovery might occur in plants that germinated rapidly (at 35°–36° C., which is near the optimum for sugar cane), as compared with those germinating slowly. It seemed possible that the greater incidence of chlorotic streak in poorly drained areas might be at least partially the result of the slower rate of germination of plants in those areas. In the case of shoots arising from infected seed cuttings, less recovery might conceivably occur because of the slower rate of development of the plants during the cool winter and spring months that follow fall planting of sugar cane in Louisiana, as a result of which the virus would invade plants developing from infected buds to a greater extent than it would in those in well-drained areas where cane germinated and grew more rapidly. In other words, rapidly growing plants might grow away from the virus. Price (11) found this to be true in tobacco plants infected with ring spot.

In a similar experiment, plants of C.P. 29/320 were grown in 6-inch clay pots of the 2 soils, with germination at the greenhouse temperature (minimum 24° C.). The 64 pots of each soil were divided into 16 groups of 4 pots each, which were randomized in their arrangement on the benches. The cuttings were planted on August 18, and the experiment was discontinued on November 17, 1942.

In addition to the 2 soils used in these experiments, muck soil was included in one series of another experiment. Composited lots of cuttings, prepared as described previously, were germinated at 22°–23° C. and 35°–36° C. in the sandy and silty clay loam soils, and at 35°–36° C. in the muck soil. The 50 plants for each treatment were divided into groups of 10, which were randomized in their arrangement on the benches. The experiment was begun on January 7 and terminated on March 17, 1944.

Germination at 35°–36° C. extended over a period of from 4 to 17 days from the date of planting, with approximately 75 per cent of the plants emerging within 5 days, and 98 per cent within 10 days. At 22°–23° C., germination extended over a period of from 8 to 59 days, with approximately 30 per cent of the plants emerging within 10 days and 76 per cent within 15 days.

The results of the 3 experiments are summarized in table 2. In each, the percentage of plants developing chlorotic streak symptoms was greater in the silty clay than in the sandy soil, and in the last experiment was greater in the muck. While the differences were not significant, either in the individual experiments or when the data were subjected to the Chi-square test, the results of other experiments which are discussed later sug-

gest the possibility that the consistently though only slightly greater incidence of the disease in the clay soil may have been related to its higher nitrogen content.[3] Even assuming that the differences in nitrogen content of the soils were great enough to affect symptom expression of the disease, however, it is, probable that in the field the differences in drainage and moisture content generally associated with the 2 soils would have a greater influence.

The rate at which the plants germinated had no measurable effect on the development of chlorotic streak, those that germinated rapidly showing symptoms to as great a degree as those that germinated slowly. In addition to determining the average percentage of diseased plants for each treat-

TABLE 2.—*Incidence of chlorotic streak in sugar-cane plants of the variety C.P. 29/320 grown from infected seed cane in sandy loam, silty clay loam, and muck soil, maintained at or near optimum moisture content*

Temperature of germination in each experiment	No. of plants in each soil	Plants with chlorotic streak			
		Sandy soil	Clay soil	Muck soil	Diff. Req'd. for P. = 0.05
		Per cent	Per cent	Per cent	Per cent
Experiment 1					
35°–36° C.	125	80.3	86.4	......	. ..
22°–23° C.	115	83.2	87.5	......	. . ..
Experiment 2					
Min. 24° C.	64	77.1	79.2	....	12.0
Experiment 3					
35°–36° C.	50	53.1	65.3	62.0	16.2
22°–23° C.	40	52.5	57.5	... .	12.1

ment, as shown in table 2, the plants were arranged in frequency classes of 5-day intervals according to the number of days required for germination, and the percentage of those showing chlorotic streak symptoms determined for each class. The averages for each frequency class were not significantly different from the general average, thus showing no relation between rate of germination and development of the disease.

Influence of Soil on Recovery from Chlorotic Streak

During the course of these studies, it was observed that chlorotic-streak-infected plants of C.P. 29/320 growing in the greenhouse in muck soil recovered from the disease to a greater extent than those growing in sandy or clay soils. Since this variety rather commonly shows recovery from the disease, experiments were conducted to determine whether the soils would have a similar effect with other varieties in which there ordinarily is less recovery.

Ten infected plants each of the C.P. numbers 29/103, 29/320, 34/139,

[3] According to unpublished analyses by Nelson McKaig, Jr., and L. A. Hurst, the nitrogen content of a typical sample of the Sharkey soil was 0.211 per cent, and of the Yazoo soil, 0.085 per cent. An unpublished analysis by R. L. Holmes gave 0.66 per cent nitrogen for the muck soil.

and 36/85, five growing in Sharkey silty clay soil and five in muck soil, were selected when 6 weeks old for uniformity of size and severity of chlorotic streak symptoms, and transferred from 6-inch pots to 4-gallon cans of the same soil where they remained for 10¼ months without the addition of fertilizer.   The stalks were then segmented into single-bud cuttings, which were planted in 4-inch pots of sandy loam soil.   The percentages of plants in each group that developed chlorotic streak symptoms were: C.P. 29/103— 53 per cent (from muck) and 91 per cent (from clay); C.P. 29/320—21 per cent (from muck) and 97 per cent (from clay); C.P. 34/139—85 per cent (from muck) and 87 per cent (from clay); C.P. 36/85—92 per cent (from muck) and 86 per cent (from clay).

Presumably the greater recovery from chlorotic streak that occurred in 2 varieties grown in muck soil was related to higher nitrogen content of that soil, the influence of which is discussed later.

NUTRITION OF THE SUGAR-CANE PLANT

According to Martin (10), the development of chlorotic streak is closely associated with the nutrition of the sugar-cane plant.   He found that the leaf symptoms disappeared when diseased plants were taken from the field and transferred to a complete nutrient solution.   He also mentioned the observations of field men that the disease was more prevalent and severe in fields of low potash content.   Bell (5, 6), on the other hand, found no indication that the disease was associated with plant food deficiencies, although he stated that unbalanced nutrition might accentuate it.   He later (7) reported no curative or preventive effects by the addition of Li, Na, Hg, Cu, B, Zn, Mn, Ba, As, Pb, or Fe to the soil.

Some experiments had indicated a relationship between nitrogen nutrition and the incidence of chlorotic streak, and since this is the principal fertilizer element applied to sugar cane in Louisiana a study of its effect on the disease was undertaken.

Nitrogen

The effect of nitrogen on symptom expression of chlorotic streak was first observed when the number of plants with the characteristic leaf streaks increased from 3 to 10 fold among 4- to 6-week-old plants of C.P. 29/320 grown from infected cuttings that had been fertilized with a solution of $NaNO_3$.   Experiments were then conducted to study the effect of the nitrogen nutrition of the sugar-cane plant on the development of the disease.

One hundred plants of C.P. 29/320 growing in 4-inch pots of sandy loam soil were divided into 2 lots after the shoots emerged.   Each pot in one lot was fertilized with 100 cc. of a solution of $NaNO_3$ containing 10 g. of the salt per liter, and the remainder were left as controls.   Notes were made on the appearance of chlorotic streak symptoms.

Sixteen plants of C.P. 29/320 growing in 6-inch pots of sandy loam soil received the equivalent of the $NaNO_3$ in the preceding experiment, but in 5 weekly applications, and an equal number was left as controls.

The results of the 2 experiments are in table 3. Additions of $NaNO_3$ were followed by marked increases in the percentage of plants showing the typical leaf symptoms of chlorotic streak. This effect became evident more slowly when the fertilizer was added in 5 applications, than when all of the nitrogen was added at one application. At the end of the experiments, when the control plants were also fertilized, they developed symptoms to practically the same extent as the originally fertilized ones, showing that the virus was present but did not become evident until the application of nitrogen caused symptoms to develop.

In other experiments it was found that $(NH_4)_2SO_4$, cottonseed meal, and filter press cake[4] as sources of nitrogen also caused an increase in the number of plants developing foliage symptoms, but the effect became evident more slowly than with $NaNO_3$.

TABLE 3.—*Comparative effect on chlorotic streak symptoms of adding equivalent amounts of $NaNO_3$ in 1 application and in 5 applications*

	Plants with chlorotic streak symptoms			
Days after fertilization	N applied once		Five applications of N	
	Fert.	Check	Fert.	Check
No.	*Per cent*	*Per cent*	*Per cent*	*Per cent*
7	20.0	8.0	6.7	12.5
14	46.0	34.0	18.7	18.7
21	60.0	48.0	56.2	25.2
48	83.0	78.0[a]	56.2	50.0[b]

[a] The check plants were fertilized 14 days prior to this reading.
[b] The check plants were fertilized 8 days prior to this reading.

The effect of nitrogen fertilization on symptom expression in plants that had already developed leaf streaks was also studied. Twenty-five 6-week-old plants of C.P. 29/320 and 10 of C.P. 28/19 with leaf streaks, growing in sandy loam soil in 6-inch pots, were fertilized and an equal number left unfertilized as controls. Another lot of 48 plants of C.P. 29/320 was fertilized, and an equal number left as controls. Notes were made on the occurrence of chlorotic streak symptoms on each leaf of all of the plants before fertilization, and again 13 days later. The results are in table 4.

The fertilization resulted in marked increases not only in the number of plants with additional streaked leaves, but also in the percentage of leaves with streaks. The new symptoms appeared more commonly on the leaves unfolded at the time of fertilization than in those unfolding subsequently.

These results raised the question as to whether the intensification of leaf symptoms following nitrogen fertilization was the result of an increase of the virus in the plant, or an accentuation of foliage symptoms resulting from movement of the virus into the leaves in response to the stimulated growth.

[4] Filter press cake is a by-product of the fabrication of sugar from sugar cane. Chemically it is a highly variable product, depending on the season, variety of cane, maturity, and fabrication methods. An average sample from Louisiana factories was estimated by Bands (13) to contain 2 per cent nitrogen.

Spencer (14) found a definite relationship between the concentration of tobacco-mosaic virus and the amounts of nitrogen supplied to plants, and (15) a marked reduction in the biological activity of the virus in plants grown in a nitrogen-deficient sand culture. It seemed desirable, therefore, to study the effect on chlorotic-streak development in sugar-cane plants of providing them with a continuously high level of nitrogen throughout their growth period, as compared with a low level. Knowledge of the influence of nitrogen fertilization on the development of the disease is of practical importance because of the custom in Louisiana of giving heavy nitrogen fertilization to plots of sugar cane being grown especially for seed cane.

TABLE 4.—*Effect of fertilization with NaNO$_3$ on intensification of chlorotic streak symptoms in plants of C.P. 28/19 and 29/320 already showing leaf streaks*

Variety	Number of plants		Chlorotic-streaked plants in which additional symptoms appeared after fertilization		Plants on which leaf symptoms first appeared after fertilization	
	Fertilized	Check	Fertilized	Check	Fertilized	Check
			Per cent	*Per cent*	*Per cent*	*Per cent*
C.P. 28/19	10	5	50.0	20.0	36.4	11.1
C.P. 29/320 ..	25	25	76.0	33.3	14.4	3.1
C.P. 29/320	48	48	52.0	20.0	33.7	6.9

TABLE 5.—*Effect of high nitrogen vs. no nitrogen on recovery from chlorotic streak in 3 varieties of sugar cane*

Variety	Leaves with chlorotic streak		Buds with chlorotic streak	
	High N	No N	High N	No N
	Per cent	*Per cent*	*Per cent*	*Per cent*
C.P. 29/103	48	47	34	52
C.P. 34/139	65	87	80	74
C.P. 29/320	38	68	22	47

Plants of C.P. 29/320 from composited lots of single-bud cuttings from infected stalks were grown in the greenhouse in 6-inch pots of Yazoo very fine sandy loam until they were 2 months old and had developed symptoms of chlorotic streak. Twenty-two were then selected for uniformity as to size and number of leaves with streaks, 11 to be fertilized with NaNO$_3$ and 11 to remain unfertilized. At this time and at intervals of 2 weeks thereafter each plant in the fertilized series was given 200 cc. of a solution containing 10 g. of NaNO$_3$ per liter. When they were 3 months old they were transplanted to 4-gallon galvanized cans of the same soil. A record was kept of leaf symptoms during the 8-month course of the experiment, when the stalks, which then had from 2 to 13 lateral buds, were segmented into single-bud cuttings and planted in the greenhouse.

In a repetition of the experiment, the varieties C.P. 29/103 and 34/139,

which ordinarily show less recovery than the 29/820, were used, with 5 plants in each of the fertilized and control series. The results of the experiments are in table 5.

The high level of nitrogen caused a marked reduction in the number of infected buds of C.P. 29/103 and 29/320. Similar results were obtained with these varieties in a field experiment, in which chlorotic-streak-infected stools of C.P. 29/320 receiving 300 lb. per acre of nitrogen as $NaNO_3$ showed apparently complete recovery, and C.P. 29/103 a significant degree of recovery in comparison with stools receiving the usual plantation rate of 40 lb. of nitrogen per acre. However, with 3 other varieties in the field test, there was no recovery.

Nitrogen and Potassium in Sand Culture

The influence of nitrogen and potassium on development of the disease was studied in sand culture. Plants of C.P. 29/320 were grown in white sea sand with the addition of a complete nutrient solution, until they were 6 to 8 inches tall, and were showing symptoms of chlorotic streak. They were then transferred to 3-gallon crocks of sand, where one series of 5 plants received a complete nutrient[5] containing 165 p.p.m. nitrogen and 208 p.p.m. potassium; a second series, 825 p.p.m. nitrogen and 208 p.p.m. potassium; and a third series, 165 p.p.m. nitrogen and 20.8 p.p.m. postassium. Changes of nutrient were made at weekly intervals, with the leachate poured back daily after making up losses from evaporation with distilled water. At the end of 5 months the stalks were harvested and the buds planted in sandy loam soil.

As in previous experiments, the high level of nitrogen caused a marked reduction in the percentage of infected buds, 35 per cent of those from this series producing diseased plants compared with 88 per cent with the lower level. The low level of potassium caused a marked increase in the occurrence of leaf streaks, approximately twice as many leaves becoming streaked in this series as in that with the normal level, and more than twice as many buds producing diseased plants. Symptoms did not disappear, however, from any of the plants grown in the complete nutrient with all elements at the normal level.

DISCUSSION

Field observations that excessive soil moisture accentuates both the incidence and severity of chlorotic streak were confirmed experimentally in the field and greenhouse. A similar response to excessive soil moisture, especially when accompanied by deficient fertilization, was reported by Jones (8) for carnation yellows, and by Pryor (12) for big vein of lettuce. Presumably, the deficiency of oxygen in waterlogged soils and possibly also the development of toxic compounds not only render the sugar-cane plant more

---

[5] The solution contained the following salts in the partial volume molecular concentrations indicated: $Ca(NO_3)_2 \cdot 4H_2O$, 0.0058; KH_2PO_4, 0.0005; $MgSO_4$, 0.001; KCl, 0.0025; with the addition of other elements in p.p.m., Fe, 5.0; Mn, 0.25; B, 0.5; Al, 0.5; Cu, 0.25; Zn, 0.25.

susceptible to infection, but favor the development of the virus in the plant after infection takes place. From the standpoint of controlling the disease, it is apparent that improvement of drainage and avoiding the planting of susceptible varieties in poorly drained soils are important.

The level of nitrogen available to the sugar-cane plant had a marked influence on the development of chlorotic streak. A dosage to young plants was followed, with all varieties studied, by the appearance of leaf symptoms earlier and in a higher percentage of the plants as compared with those receiving no nitrogen. Likewise, single high-nitrogen applications to older plants, particularly if they had begun to show indications of nitrogen deficiency, resulted in the appearance of leaf symptoms in many plants previously symptomless, as well as intensification of symptoms in those already streaked. Continuing the heavy nitrogen applications at intervals throughout the life of the plant, on the other hand, eventually caused a decrease in the percentage of infected buds of 2 varieties studied in the greenhouse, and apparently complete recovery in one of them in a field trial. With other varieties, however, there was no significant degree of recovery.

The level of nitrogen required to induce recovery was much higher than would ordinarily be applied to sugar cane in the field, even to that being grown for seed cane. It is doubtful, therefore, whether the amounts of nitrogen commonly used in plantation practice would have any material effect on the prevalence or severity of the disease.

One possible explanation of the recovery that occurred is that in the plants receiving the continuous high level of nitrogen the virus did not multiply in proportion to the rate of growth of the plants. The increase in symptoms resulting from the single large dosage of nitrogen may have been the result of the movement of the virus into the leaves in response to the stimulated growth. Then, in those plants that were supplied with additional nitrogen and continued to grow rapidly, the virus did not increase proportionately so that the ultimate effect was a dilution of the concentration present, resulting in a decrease in the percentage of infected leaves and buds, or in those with sufficient concentration of virus for the characteristic symptoms to become manifest.

While marked deficiencies of a critical element accentuated the severity of chlorotic streak, the affected plants did not necessarily recover from the disease when the deficiencies were corrected. This did not occur in these experiments even with C.P. 29/320, a variety in which there often is considerable recovery from the disease. Judging from the literature and from the results of experiments reported in this paper, the occurrence of the disease is apparently not associated specifically with the deficiency of any particular element but may be accentuated by poor growth conditions resulting from several causes.

SUMMARY

Chlorotic streak developed to a greater degree in both the field and greenhouse in sugar-cane plants grown from infected seed cuttings in poorly

drained soil than in comparable soil with good drainage, and secondary spread of the disease was greater in plants grown from healthy seed cuttings in the field under conditions of poor drainage as compared with good drainage.

In the greenhouse, there was no significant difference in incidence of the disease among plants grown from infected seed cuttings in Sharkey silty clay and Yazoo very fine sandy loam soils.

Applications of nitrogenous fertilizer to young plants grown from infected cuttings increased the number developing leaf symptoms. Large dosages of nitrogen at frequent intervals during the life of the plants caused a high degree of recovery in two varieties in the field and greenhouse, but had no significant effect on other varieties studied.

Recovery in the absence of added nitrogen was greater in plants of two varieties grown in muck soil than in clay soil, which was attributed to the higher nitrogen content of the former soil.

DIVISION OF SUGAR PLANT INVESTIGATIONS,
    U. S. DEPARTMENT OF AGRICULTURE,
      HOUMA, LOUISIANA.

LITERATURE CITED

1. ABBOTT, E. V. A progress report on the study of chlorotic streak of sugar cane in Louisiana. Sugar Bull. 18(19): 3–5. 1940.
2. ———————— and J. W. INGRAM. Transmission of chlorotic streak of sugar cane by the leaf hopper *Draeculacephala portola*. Phytopath. 32: 99–100. 1942.
3. BELL, ARTHUR F. 34th Ann. Rept. Queensland Bur. Sugar Exp. Stations, pp. 50–69. 1934.
4. ————————. 35th Ann. Rept. Queensland Bur. Sugar Exp. Stations, pp. 39–48. 1935.
5. ————————. 37th Ann. Rept. Queensland Bur. Sugar Exp. Stations, pp. 34–41. 1937.
6. ————————. 38th Ann. Rept. Queensland Bur. Sugar Exp. Stations, pp. 41–50. 1938.
7. ————————. 42nd Ann. Rept. Queensland Bur. Sugar Exp. Stations, pp. 11–13. 1942.
8. JONES, LEON K. Mosaic, streak, and yellows of carnation. Phytopath. 35: 37–46. 1945.
9. MARTIN, J. P. Chlorotic streak disease of sugar cane. Proc. 5th Cong. Internat. Soc. Sugar Cane Techn., pp 823–829. 1935.
10. ————————. Rept. of the 56th Ann. meeting of the Hawaiian Sugar Planters Assn., p. 30. 1937.
11. PRICE, W. C. Virus concentration in relation to acquired immunity from tobacco ring spot. Phytopath. 26: 503–529. 1936.
12. PRYOR, DEAN E. The big vein disease of lettuce in relation to soil moisture. Jour. Agr. Res. [U.S.] 68: 1–9. 1944.
13. RANDS, R. D. Filter press cake reduces damage from root diseases in black lands. Sugar Bull. 11(1): 2–4. 1933.
14. SPENCER, E. L. The effect of host nutrition on concentration of tobacco-mosaic virus. Plant Physiol. 14: 769–782. 1939.
15. ————————. Inhibition of increase and activity of tobacco-mosaic virus under nitrogen-deficient conditions. Plant Physiol. 16: 227–239. 1941.

CERCOSPORA LEAF SPOT OF BROAD BEAN IN CHINA

T . F . Y u [1]

INTRODUCTION

In the broad-bean growing regions of southeastern China, the crop is attacked by a leaf-spotting fungus commonly referred to as *Cercospora fabae* Fautrey. The disease is rather common, but generally is of little economic importance except in the low, poorly drained rice land where bean is rotated with rice. In this case, leaf spot first appears on the dense lower leaves of the plant in early spring. Until March or April, the infected lower leaves, especially during wet weather, either decay or drop and the normal development of the plant is suppressed. Infected plants usually are less prolific than healthy ones.

REVIEW OF LITERATURE

Cercospora fabae Fautrey was first described by Fautrey in 1890 on broad bean collected at Clamercy, Côte-d'Or (2). It has since been reported as occurring in China, Bohemia, France (9), Japan (8), England (5), Cyprus (3), and Italy (6).

Apparently very little work has been done on *Cercospora* leaf spot of broad bean. Woodward (12), in 1932, described in detail the symptoms of the disease, the morphology of the causal fungus, and the results of inoculations. As far as the writer is aware, that is the only paper that reports a comprehensive investigation of this disease.

SYMPTOMS

In the field in China, the disease appears primarily on leaves, only rarely on leaf petioles and stems, and never on flowers and pods.

The first symptom is a chocolate-colored spot about 1 mm. in diameter on the lower leaves. At the end of spring season, spots are seen on the upper leaves. Under favorable conditions, spots enlarge rapidly. The center of a spot becomes light gray with a broad, slightly raised, deep red margin. The lesions are usually zonate. The number of spots on a single leaflet varies from 1 to 20, usually 1 to 4. Spots are oblong, round or occasionally irregular, from 1 to 14 mm. in diameter, the average falling between 5 and 7 mm. These spots may occur on any part of the leaf surface, including tips and margin, and they are not limited by veins.

The appearance and size of the spots vary with climatic conditions. In dry seasons, the spot remains small with depressed center and raised, deep red ridge. The larger spots have distinct margins. In a wet climate, the black central area of the spot extends rapidly and the margin becomes less and less distinct (Fig. 2, B). Numerous conidia are then produced on the

[1] Formerly Professor of Plant Pathology, The University of Nanking, Nanking, China.

center of the spot (Fig. 2, A), which then appears silver gray.   Frequently the dead tissue falls out, leaving a shot-hole effect on the leaf.

If several spots are on the same leaf blade, the tissue between them may collapse to form a large dead area.   Sometimes, the veins on which spots are located become discolored and the leaf tissue beyond the vein has a tendency to wilt and soon turn black.   Large, irregular, dead areas formed by coalescence of the spots may cover a large portion of the leaf surface.   When the spots occur on the tip or margin of a leaf the dead tissue soon falls out.   The various symptoms of the disease on leaves are shown in figure 1.

FIG. 1.   Spots produced by *Cercospora fabae* Fautrey on the leaves of *Vicia faba* L. in the field.

Only in rare cases, does the fungus produce lesions on leaf petiole, stipule, and stem.   The lesions on leaf petiole and stem are spindle-shape, or oblong, with gray center and deep red margin, almost indistinguishable from the young lesions produced by *Ascochyta fabae* Speg.   During wet seasons, black lesions on these plant parts are not uncommon.   Sporulation of the fungus has not been observed on them.

THE CASUAL FUNGUS

Two species of *Cercospora* have been reported on broad bean.   One is *C. fabae* described by Fautrey (2) and the other is *C. zonata* described by Winter (11).   They presumably differ from each other in size and septation of conidia and in color of conidiophore.   These characters are, however,

by no means constant. Welles (10) pointed out that conidia of some species of *Cercospora* may vary in size and septation under different environments. Woodward (12) examined conidia collected from the field in England. Their dimensions were 40–126 × 4–6 μ with an average of 75 × 4.7 μ. The number of septa varied from 3 to 7, the average number being seven. He also showed that conidia from plants grown in a cool greenhouse averaged 93 × 4.5 μ. Their range was 44–171 × 3.5–5.5 μ. The number of septa varied from 1 to 14, the average being seven. Because size and septation of conidia are variable characteristics of the species of *Cercospora*, Woodward believes that *C. fabae* Fautrey and *C. zonata* Wint. may be identical. The writer also found that conidia of *C. fabae* Fautr. vary in size and septation under different environments. The fungus responsible for the leaf-spot disease on broad bean in China therefore is identified as *C. fabae* Fautr.
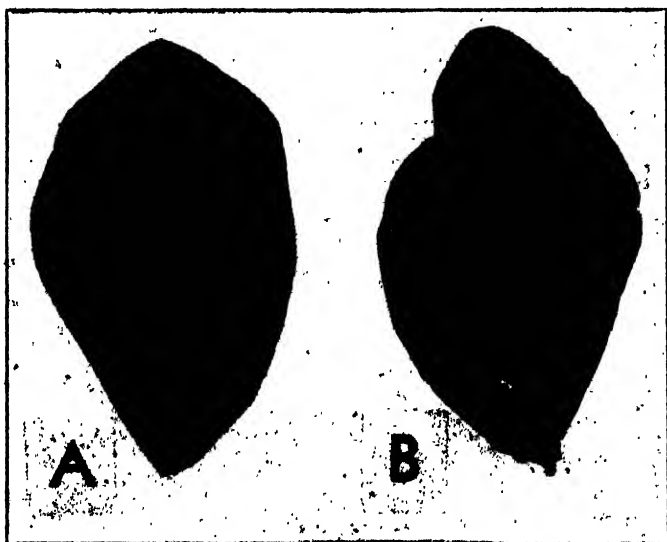
FIG. 2. Cercospora spots on leaves under moist conditions. A. Spot with sporulation and indistinct margin. B. Spot becoming soft and black, the margin of the spot becoming less distinct.

The conidiophores of the present fungus emerge in clusters of 1 to 12, mostly 3 to 6, from the leaf surface. They are brown, 0 to 2 septate, usually simple, and 15.8–99.4 × 4.5–6.0 μ. Conidia, borne terminally, are elongate, tapering above, straight or slightly curved, obclavate, and colorless. Conidia collected from the field measured 29.2–102.8 × 2.8–4.6 μ with an average of 56 × 3.8 μ. The number of septa varies from 2 to 9, the average being seven. The size of 312 conidia, collected from beans grown in a damp rice field, was 34.4–131.7 × 3.2–5.0 μ with an average of 64 × 4.2 μ. The number of septa varied from 1 to 15, the average being nine.

INOCULATION EXPERIMENTS

Pathogenicity of the fungus has been determined by inoculation ex-

periments in the greenhouse. Young and half-grown broad bean plants were inoculated with a water suspension of conidia obtained from a diseased plant grown in the greenhouse. In the present experiments, tiny, water-soaked, red spots appeared 2 to 3 days after inoculation. It took about 10 to 15 days to produce the typical zonate spots characteristic of field infections. Numerous experiments of this sort showed that conidia of the fungus can infect the noninjured leaves of broad bean. It is of interest that Woodward (12) was unable to infect noninjured leaves in his inoculation experiments.

Inoculation of the leaf petiole and stems with suspensions of conidia in the greenhouse occasionally resulted in tiny red spots. The results of these experiments, however, are inconsistent. Apparently, under normal conditions, the fungus does not infect these plant parts.

HOST RANGE

Numerous inoculations were made to determine the host range of the fungus. Leaves of plants to be inoculated were washed with mercuric chloride (1–1000) and rinsed with sterile water. Spore suspension was then sprayed on the upper surface of the leaf. After inoculation, the plants were kept in a moist chamber 48 hr. and then set on greenhouse benches. The fungus was not able to infect the following species of plants: *Pisum sativum* L., *P. sativum* var. *arvense* Poir., *Lens esculenta* Moench, *Vicia sativa* L., *V. cracca* L., *V. villosa* Roth., *Lathyrus odoratus* L., *Phaseolus vulgaris* L., *Vigna sinensis* Endl., *Dolichos lablab* L., *Glycine max* Merr., *Medicago sativa* L., *Melilotus alba* Desr., *Trifolium repens* L., and *T. pratense* L.

CULTURAL CHARACTERS

The fungus has been grown on most of the common culture media. Potato-dextrose agar seemed to be most favorable for its growth. In Petri dishes, the fungus was first white and gradually became light gray. As growth proceeded, the coloring became dark gray to black. The center of the colony was usually slightly raised and at the margin there might be one or two zones present. Sometimes, irregular dark gray or deep olivaceous green patches were on the surface of the colony. In old cultures, the mycelium became dense and compact. Sporulation of the fungus in artificial culture media, by means of the methods suggested by Nagel (4) and Diachun and Valleau (1), could not be induced.

TEMPERATURE RELATIONS

The relation of temperature to fungus growth was judged by the increase in diameter of fungus colonies on agar plates. Each 90-mm. Petri dish contained about 15 cc. of one per cent potato-dextrose agar. Single spores were isolated from the fungus, and small bits of mycelial growth of approximately equal size from young, vigorous colonies were used to plant test plates. Quadruplicate plates were placed in constant-temperature chambers

held at 5°, 10°, 16°, 25°, 27°, 31°, and 33° C. The average diameter of
colony was determined at 24-hr. intervals.

Based on the radial growth of the fungus during 6 days at different
temperatures, the optimum was about 25° C., with a maximum slightly
over 31° C. and a minimum about 5° C. Cultures grew when kept at 5° C.
for a long time.

OVERWINTERING

In May, 1928, severely diseased leaves were buried one inch beneath the
soil surface, between wire screens. In January, 1929, the decayed leaves
with adhering soil were dug out and laid over a large wooden box of soil
in which broad-bean seeds had been sown. These boxes were covered by a
moistened cloth tent for two days and were set in a cool greenhouse. Suffi-
cient water was added to keep them moist. On February 15, the first
symptom of the disease appeared on the lower leaves. Typical *Cercospora*
spots were on most of the leaves at the end of the month. A quantity of the
decayed leaves with adhering soil was brought into the laboratory. It was
soaked in 200 ml. sterile water and the mixture was shaken vigorously for a
few minutes. After standing for about 20 min., the supernatant water was
centrifuged. Numerous fresh conidia were thus collected. When sprayed
on healthy broad-bean seedlings, the conidia caused infection. Microscopic
examination of the decayed leaves revealed that either conidiophore clusters
or stromatic mycelium of the fungus remained alive throughout the winter.
Conidiophores might produce fresh conidia for the primary infection in
the early spring when conditions were favorable. When the experiment
was repeated in 1933, the result was the same. .

In other experiments, diseased leaves were collected in May, 1934, and
dried. They were pulverized and kept in cool places. In winter, they were
laid about ¼-inch thick over the surface of soil in which broad-bean seed-
lings were growing. The typical disease developed.

These experiments show that the fungus overwinters in diseased leaves.

SUMMARY

A leaf spot of broad bean caused by *Cercospora fabae* Fautr. is very
prevalent in southeastern China. The disease is serious in wet seasons in
the lower rice land where beans are rotated with rice. In this case, the
lower leaves decay and drop in early spring and thus check the normal
development of the plant. In the well-drained rice fields and on the uplands
the disease is of little economic importance.

Spots, primarily on leaves, are oblong, round, or irregular, mostly 5 to 7
mm. in diam., gray at center, chocolate to red along the margin, and zonate.
The diseased tissue may fall out, leaving a shot-hole. Under moist condi-
tions, the infected lower leaves of the plant may either fall or decay.

Inoculations in the greenhouse with suspensions of the conidia show that
they may infect noninjured leaves of broad bean. The fungus is confined to

Vicia faba L. and is unable to infect 14 species and one variety of leguminous plants tested.

The fungus overwinters on diseased leaves as conidiophores or stromatic mycelium.

THE INSTITUTE OF AGRICULTURAL RESEARCH,
    TSING HUA UNIVERSITY, CHINA.

LITERATURE CITED

1. DIACHUN, STEPHEN, and W. D. VALLEAU. Conidial production in culture by *Cercospora nicotianae*. Phytopath. 31: 97–98. 1941.
2. FAUTREY, F. in C. ROUMEGUERE. "Fungi exsiccati precipue Gallici. Centurie LVI°", No. 5588. Rev. Myc. 13: 13. 1891.
3. NATTRASS, R. M. Annual report of the mycologist for the year 1935. Rep. Dir. Agric. Cyprus 1935: 57–64. 1936.
4. NAGEL, CLATUS M. Conidial production in species of *Cercospora* in pure culture. Phytopath. 24: 1101–1110. 1934.
5. PETHYBRIDGE, G. H. Fungus and allied diseases of crops, 1925, 1926, and 1927. Gt. Britain Min. Agr. and Fish., Misc. Publ. 70. 1929.
6. PETRI, L. Rassegna dei casi fitopatologici osservati vel 1927. Boll. R. Staz. Pat. Veg. 8: 1–50. 1928.
7. RADEMACHER, B. Erfahrungen uber die wichtigsten Krankheiten der Ackerbohne und Bekampfung. Deutsche Landw.-Presse 61: (21) 253–254, (22) 275–276, (23) 296. 1934. Abstr. in Rev. Appl. Myc. 13: 670. 1934.
8. SHIRAI, M. and K. HARA. A list of Japanese fungi hitherto known. 2nd Ed., Tokyo, Japan. 1917.
9. STEVENSON, J. A. Foreign plant diseases. U. S. Dept. Agr. 198 pp. 1926.
10. WELLES, COLIN G. Observations on taxonomic factors used in the genus *Cercospora*. Science 59: 216–218. 1924.
11. WINTER, G. Contributiones ad floram mycologicam lusitanicam. Ser. 5. Bol. Soc. Broter II, 1883: 49. 1884.
12. WOODARD, R. C. *Cercospora fabae* Fautrey, on field beans. Trans. Brit. Myc. Soc. '17: 195–202. 1932.

HELMINTHOSPORIUM ROSTRATUM ON CORN, SORGHUM, AND PEARL MILLET[1]

GEORGE Y. YOUNG, C. L. LEFEBVRE, AND A. G. JOHNSON[2]

In 1927 and again in 1928, Bunting[3, 4] reported a species of *Helmintho-sporium* on corn (Maize) that was considered very close to *H. rostratum* Drechs. His figures show the characteristic form of spores, including the rostrate tips and prominently protruding hila, but there is no indication or

FIG. 1. Conidia of *Helminthosporium rostratum* isolated from Pearl millet collected at Tifton, Ga., 1944. (× 360.)

[3] Bunting, R. H. Local cereal diseases in the records of the Mycological Division. Dept. Agr. Gold Coast Yearbook 1926, Bul. No. 7: 25–27. 1927.
[4] Bunting, R. H. Black auricle disease of maize. *In* Fungi affecting graminaceous plants of the Gold Coast. Dept. Agr. Gold Coast, Bul. 10: 19–20. 1928.

description of the dark, thickened terminal septa, so characteristic of this species. These may have been overlooked, however, or their importance as a diagnostic character underestimated. Dade[5] lists the fungus reported by Bunting as "*H.*(?) *rostratum* Drechsl.*" and cites both of Bunting's reports without comment. In 1941, Lefebvre and Johnson[6] listed *H. rostratum* Drechs. on *Zea mays* L. collected by Lefebvre at Tifton, Ga., in 1940.

In the summer of 1944, a fungus that was clearly *Helminthosporium rostratum* Drechs. was isolated from leaf spots on corn (*Zea mays* L.) from State College, Miss. (Young, Cult. No. 2311), and Tifton, Ga. (Lefebvre, Cult. No. 1847); on Honey sorghum (*Sorghum vulgare* Pers., Cult. No.

FIG. 2. Lesions on leaves of corn (Inbred Fla. 22) following artificial inoculation with *Helminthosporium rostratum* in the greenhouse at Beltsville, Md., January 1945.

1615), Spur feterita (*S. vulgare*, Cult. No. 1613), Common and Sweet Sudan grass (*S. vulgare* var. *sudanense* (Piper) Hitchs., Cult. No. 1679) from Gainesville, Fla.; and on Pearl millet (*Pennisetum glaucum* (L.) R. Br., Cult. No. 1657) from Tifton, Ga.

While there were slight differences in the spores from the various hosts, the essential characters agreed with those given by Drechsler[7] for the species as originally described from *Eragrostis ciliansis* (All.) Link (*E. major* Host), including elliptical and rostrate tipped spores, the latter with dark, protruding hila, and dark, thickened, distal and basal septa (Fig. 1).

There is a considerable variation in the lesions on the leaves of the various

[5] Dade, H. A. A revised list of Gold Coast fungi and plant diseases. Kew Roy. Bot. Gard. Bul. Misc. Inform. No. 6 (1940): 205–247. 1940.
[6] Lefebvre, C. L., and H. W. Johnson. Collections of fungi, bacteria, and nematodes on grasses. U. S. Dept. Agr., Plant Dis. Rptr. 25: 556–579. 1941.
[7] Drechsler, Charles. Some graminicolous species of *Helminthosporium*: I. Jour. Agr. Res. [U.S.] 24: 722–724. 1923.

host plants. In general, the lesions are small, 1–2 × 2–5 mm., and commonly limited laterally by the leaf veins. Lesions may coalesce, however, to form larger, necrotic areas, which extend across the veins. The necrotic centers of all the lesions, particularly on corn, bleach to straw color, usually with slight browning at the edges and some yellowing at the ends.

On sorghums and sorghum relatives there is more or less purpling except on Leoti sorgo, and Tift and Sweet Sudan grass. On Pearl millet, the lesions at first are rather dark brown and later tend to become light brown, particularly on the older leaves.

ARTIFICIAL INOCULATIONS

In 1945, corn in Mississippi, and at Beltsville, Md., was inoculated with isolates No. 2311 and No. 1847. Both isolates infected corn (Fig. 2) and the fungus was re-isolated from the lesions.

TABLE 1.—*Disease ratings on plants of corn, sorghum and sorghum relatives, and Pearl millet following inoculation in the greenhouse at Beltsville, Md., with cultures of Helminthosporium rostratum from various sources, 1946*

Plants inoculated	Disease ratings following 2 series of inoculations with culture No.											
	2311		1847		1615		1613		1679		1657	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
Corn												
Fla. 4	2	6	1	5	4	7	5	5	2	5	4	7
Fla. 6e–23	2	2	1	4	2	6	3	5	1	6	3	5
Fla. 21e–2	2	7	4	5	5	6	6	5	5	5	6	8
Fla. 22	1	6	1	5	2	6	3	6	1	5	3	7
Yellow Paymaster	5	5	2	5	2	5	2	6	4	4	5	6
Sorghum etc.												
Honey	1	1	1	1	2	2	1	2	1	2	2	3
Leoti sorgo	0	1	0	1	1	1	0	1	0	1	1	1
Spur feterita	1	2	0	2	1	3	1	4	1	2	1	3
Red x	1	1	1	1	1	2	1	1	1	2	2	2
Acc. No. 5	2	2	1	2	2	3	2	2	2	3	3	3
Common Sudan grass	1	2	2	2	2	2	3	2	2	4	2	4
Sweet Sudan grass	1	1	1	1	1	1	1	1	0	1	1	1
Tift Sudan grass	1	1	1	1	1	1	2	1	0	1	2	1
Johnson grass	2	2	1	1	1	3	3	2	1	2	4	3
Pearl millet												
Commercial	0	6	0	3	0	8	0	7	0	8	0	7
Sel. 794	1	6	0	1	0	5	0	7	0	6	0	6

In January, 1946, single-spore cultures from the six sources were used in two series of cross-inoculations at the Plant Industry Station, Beltsville, Md.

The cultures, grown on carrot decoction with dextrose added, were homogenized in a Waring Blendor for two minutes and atomized on the plants, after which the plants were left in a moist chamber for 48 hours. Following this the plants were placed in a greenhouse at about 80° F. and kept well watered.

Plants in series A were inoculated January 28, 1946, when fairly young, that is, from 8 to 10 inches tall. The disease ratings were made February 1. For series B, older leaves of the same plants were reinoculated with the same

cultures, respectively, the latter part of March and disease ratings were made April 1.   This procedure was possible as there had been no spread of infection to the later leaves.

The disease ratings were made on the basis of severity of infection on practically all leaves from 0, no infection, to 10, very abundant infection, and various gradations between.   Ratings below 3 indicate high resistance.

The plants inoculated and the disease ratings in both series A and B are given in table 1.   The most severe infections were obtained on corn and Pearl millet; none of the sorghums or sorghum relatives tested were very susceptible. Leoti sorgo was the most resistant.   Sweet and Tift Sudan grass, both of which originated as hybrids between Leoti sorgo and Common Sudan grass, likewise were highly resistant.   Pearl millet was susceptible only in the older stage (series B).   Usually corn was more susceptible in the older stage (series B) than in the younger stage (series A).

While there were some indications of differences in the pathogenicity of the cultures from the different sources, this phase needs additional study before definite conclusions are justified.

SUMMARY

Helminthosporium rostratum Drechs. has been isolated from leaf spots on corn from Mississippi, and Georgia; on sorghum and sorghum relatives from Florida, and on Pearl millet from Georgia.

Six cultures from the above sources were pathogenic on these various hosts in cross-inoculation experiments.

PLANT INDUSTRY STATION,
    BELTSVILLE, MARYLAND,
        AND
MISSISSIPPI AGRICULTURAL EXPERIMENT STATION.

THE EFFECT OF SEEDLING DISEASES OF CASTOR BEANS ON THE SUBSEQUENT PLANT DEVELOPMENT AND YIELD

E. C. Stevenson[1]

Seedling diseases in commercial plantings of castor beans in the United States have frequently resulted in very poor stands and probably have been an important cause of the low yields that are reported at times for this crop. The damage to the seedlings varies from cases in which the cotyledons are malformed, spotted, and stunted to those in which the seedling is killed. *Alternaria ricini* (Yoshii) Hansford, species of *Fusarium*, and various

FIG. 1. Seedling blight and die-back of castor bean. The seedling in the center has not been affected while those to the right and left have a common type of injury.

Mucorales have been found by extensive isolation studies to be most frequently associated with these malformed cotyledons and seedling die-back of castor beans at Beltsville, Maryland (Fig. 1).

When only portions of the cotyledons are affected and the growing point is not involved, the seedlings recover and, after the cotyledons have abscissed, the plants apparently develop normally for the remainder of the season. Because such infections of the cotyledons usually occur each year and have

184

been very widespread in some plantings, a study was made during the seasons of 1943 and 1944 to determine the effect of this injury on the subsequent development of the affected plants that survived.

MATERIALS AND METHODS

Seed of the varieties Conner, Kentucky 38, and Doughty 11 were used in the two-year study. The experimental design was a split-plot randomized-block of four replications with varieties occupying the main plots. Each sub-plot contained four rows of ten hills each; only the two center rows being used as data rows. Twelve to 14 seeds were planted per hill and each hill was thinned to one plant before the first true leaves were well formed. The thinning was done in such a manner that two classes of plants made up the sub-plots. These were the "control" class, made up of plants selected at the time of thinning because they were apparently disease-free, and, the

TABLE 1.—*The effect of seedling diseases of the castor-bean plant on seedling survival and the yield of seed per plant in 1943 and 1944 at Beltsville, Maryland*

Comparisons	Average number of plants that survived per plot of 20 plants		Average yield of seed per plant	
	1943	1944	1943	1944
			Grams	*Grams*
Class (all varieties)				
Injured	14.9	19.2	155.93	113.40
Controls	18.6*	19.8	158.76	136.08*
Variety (both classes)				
Conner	17.5	19.7	167.27	127.58
Kentucky 38	16.9	19.0	170.10	119.07
Doughty 11	16.0	19.8	136.08	124.74

* Significantly greater than the corresponding figure for the injured class at the 5 per cent level.

"injured" class containing plants that were selected as being diseased at the time of thinning. Data were taken on the following: (1) number of plants that survived per plot, (2) number of racemes per plant, (3) number of racemes harvested, (4) yield per raceme, (5) yield per plant, (6) yield per plot, (7) hulling percentage,[2] and (8) test weight per bushel. By applying the F test to the error mean squares for each item for the two years it was found that the data were in sufficient agreement to permit a combined analysis for the two years except in the cases of items (1) and (5) where separate analyses were made for each year of the test.

RESULTS AND DISCUSSION

Data on the number of plants that survived per plot and the average yield per plant are presented in table 1. In 1943 there was a statistically significant loss of plants per plot in the injured class because many of the seedlings died subsequent to thinning. Consequently, in thinning the plots

[2] Hulling percentage is that proportion of hulled, sound seed obtained from a given weight of air-dry seed in the hull.

in 1944 only those injured seedlings were left which appeared as if they would survive the disease, thereby making the comparison between the two classes more equable.   No significant difference in the number of plants which survived per plot occurred in 1944 when this more selective method of thinning was used.   The difference in stand between the two years is reflected in the average yields per plant as shown in the table.   In 1943 there was no difference in the average yield per plant between the two classes while in 1944 the individual plant yields in the injured class were significantly less than those of the controls.   This apparently was due to the reduced competition between plants in the injured class (since there were fewer plants per plot)

FIG. 2.   Reduction in early vigor of castor beans (Conner variety) caused by seedling blight.   The plants in the four rows left of center had diseased cotyledons while those to the right of center were not affected.

in 1943 resulting in the production of more seed per plant.   In 1944 there was no difference in stand and individual plants in the injured class produced significantly less seed per plant than the controls.

It was obvious immediately after emergence and until mid-season in both years that the plants from diseased seedlings were less vigorous than the controls (Fig. 2).   Later this effect was not so pronounced, although the vigor of the former never reached that of the latter.

A summary of the combined analysis for 1943 and 1944 of the other characters studied is presented in table 2.   In both years significantly fewer racemes were harvested per plant and per plot from the injured class than from the control class.   In the first year this was partially due to the reduced stand of injured plants, but in both years it was caused primarily

by the fact that plants from affected seedlings matured somewhat later and therefore correspondingly fewer racemes from these matured before frost. In the first year the injured plants of the three varieties (Conner, Kentucky 38, and Doughty 11) produced a total yield of seed per plant which on the average was 26.4, 14.4, and 28.7 per cent less, respectively, than that of the control plants. In 1944 the figures for Conner and Kentucky 38 were 35.6 and 18.5 per cent, respectively. In the case of Doughty 11 there was no difference in the yields from the injured and control plants in 1944 but at the time of thinning the number of injured seedlings of this variety was very small, and after thinning the injured plants were therefore much like the controls.

TABLE 2.—*Summary of the effect of seedling diseases of the castor bean on plant development and seed yield. (Each figure is an average of four replications and two years)*

	Racemes harvested per plot	Av. No. racemes harvested per plant	Av. yield of seed per raceme	Seed yield per plot	Calculated yield per acre	Hulling percentage	Weight per bushel
	Number		*Grams*	*Grams*	*lb.*		*lb.*
Class (varieties combined)							
Injured	72.3	4.3	37.42**	2221	870	68	42
Controls	97.0**	5.0*	32.89	2804**	1098**	69	43
Variety (classes combined)							
Conner	61.0	3.3	47.06**	2713	1063	69	42
Kentucky 38	125.6**	7.0**	20.13	2514	985	69	41
Doughty 11	67.2	3.7	38.19**	2311	905	68	45

* Significantly greater than the corresponding figures at the 5 per cent level.
** Significantly greater than the corresponding figures at the 1 per cent level.

The control plants yielded significantly less seed per raceme than the injured plants in both years. This is explained by the fact that the injured plants matured later and the bulk of the yield from this class was obtained from the first or main racemes which usually are much larger than the later racemes in the case of these varieties. In the control plots many of the smaller, later-formed racemes ripened in time to be harvested, thus lowering the average yield per raceme.

The quality of the seed from the two classes of plants was the same since there was no significant difference in hulling percentage and the weight per bushel.

The three varieties did not differ significantly in yield, hulling percentage, and weight per bushel. Kentucky 38 produced significantly more racemes per plot and per plant than either of the other varieties, while Doughty 11 and Conner yielded significantly more seed per raceme than Kentucky 38.

SUMMARY

Seedling infections by various fungi resulting primarily in cotyledonary injury of castor-bean plants had a pronounced effect on the subsequent plant growth and yield even when the affected plants recovered. Affected seedlings that were not killed were retarded more or less in proportion to the extent of the infection. This was indicated by a reduction in height and general vigor as well as by later maturity. The yields of seed in the two years studied were markedly reduced by seedling infections because of the reduced vigor and later maturity. The quality of the seed produced from plants with cotyledonary injury did not seem to be affected as there was no significant reduction in either the hulling percentage or weight per bushel.

PLANT INDUSTRY STATION,
    BELTSVILLE, MARYLAND.

PHYTOPATHOLOGICAL NOTES

Poria obliqua on Dying Beech.—*Poria obliqua* (Pers.) Karst. is an important wood-decay organism found principally on species of *Betula*, occasionally on *Ostrya* and *Fagus*. On live hosts it is reported to form only rimose, clinker-like masses of sterile tissue commonly known as sterile conks. Fertile sporophores, apparently, were believed to be produced only on dead trees and only after decay was well developed.[1]

In the vicinity of Tully, New York, August, 1946, a fertile fruit-body of *Poria obliqua* was found on a dying trunk (d.b.h. 5 inches) of *Fagus grandifolia* Ehrh. A part of the lower trunk was alive as evidenced by a living branch, soundness and high moisture content of a portion of the sapwood of the trunk, green cortical cells, and the presence of demonstrable nuclei in the parenchyma of the sound sapwood.
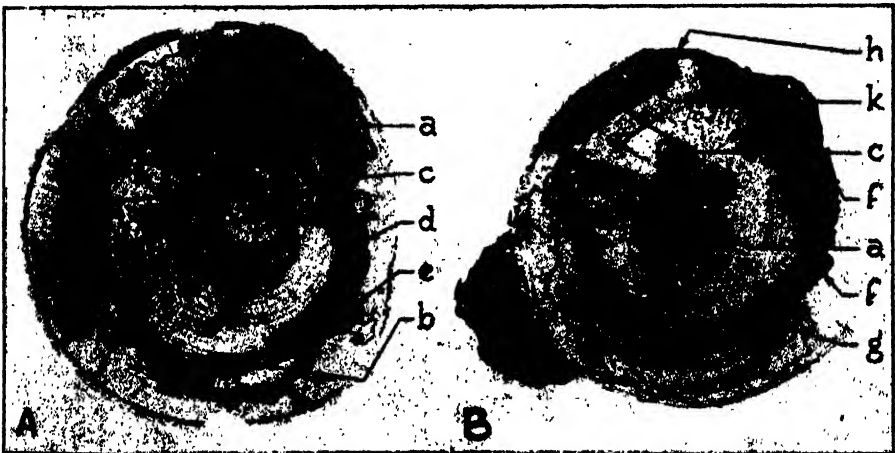
Fig. 1. Cross sections of the trunk of a dying beech on which a fertile sporophore of *Poria obliqua* was formed. A. A section taken directly below the resupinate sporophore. × ⅔. B. A section taken 15 inches above Section A and through the sporophore. × ⅔. a, advanced stage of decay; b, intermediate stage of decay; c, living sapwood; d, black zone line; e, concentrations of hyphae behind the black zone line; f, sporophore; g, mat of mycelium which developed behind the black zone line; h, sporophore tissue adjacent to living sapwood; k, cinnamon brown zone line adjacent to living sapwood.

The portion of the beech stem bearing the fertile fruit-body in close association with living host tissue was cut into sections for study. In cross sections of the trunk (Fig. 1) black zone lines were common to the sapwood and separated advanced decay from earlier stages of decay. In areas where the sapwood was still living the zone lines were cinnamon brown,[2] less distinct, and considerably wider than elsewhere. Microscopic examinations of thin sections through the zone lines disclosed that the lumina of many

[1] Campbell, W. A., and Ross W. Davidson. A Poria as the fruiting stage of the fungus causing the sterile conks on birch. Mycologia 30: 553–560. 1938.

[2] Ridgway, R. Color standards and color nomenclature. 43 pp. 53 color plates. Washington, D. C. 1912.

cells were filled with swollen brown hyphae, whereas others appeared to contain an amorphous dark-colored substance.   A thin mat of mycelium, consisting of brown, heavy-walled hyphae, was present at the edges of the sporophore a few millimeters nearer the heartwood than the black zone line.   As this mat of mycelium thickened it apparently loosened a shell of sapwood beneath which the fertile sporophore developed.   As the fruit body formed, decayed sapwood and sound sapwood as much as 10 millimeters in thickness were forced off.   The sporophore was in intimate contact with the living sapwood.

The presence of the fertile form of *Poria obliqua* on a tree not dead suggests that live trees bearing sterile, rimose conks might advisably be removed from the forest during sanitation cuttings.—ROBERT A. ZABEL, Department of Forest Botany and Pathology, The New York State College of Forestry, Syracuse, New York.

The Observed Frequency of Mature Pycnidia of Septoria gladioli on Gladiolus Corms.—Such references as appear in the literature dealing with the presence of mature pycnidia of *Septoria gladioli* Pass. on the corms of *Gladiolus spp.* emphasize the rare occurrence of this stage.   Massey,[1] who first demonstrated the connection of the hard rot of the corms with the leaf spot caused by this pathogen, stated that he failed to find pycnidia on the corms.   Pape,[2] who first noted mature pycnidia on hard rot lesions, gives no data on their frequency.   Moore[3] writes that he found this fruiting stage "only on three occasions in the last twelve years during which time a very large number of corms affected with Hard Rot have been examined."

The situation came to the writer's attention following the interception of pycnidia with mature conidia on a gladiolus corm in January, 1946, and led to an examination for the mature pycnidial stage in shipments of gladiolus which passed through the Bureau of Entomology and Plant Quarantine inspection house at Hoboken, N. J.

One hundred and fifty shipments examined between January 14 and June 15, 1946, had hard rot lesions.   The pycnidial stage bearing mature conidia of *Septoria gladioli* was on 10 of these shipments.   This number represents 6.6 per cent of the shipments infected with typical hard rot lesions.

The corms bearing the fruiting pycnidial stage came from Holland, England, Australia, and Canada.

Mature pycnidia are to be sought on corms with an advanced stage of the disease.   This may be indicated by the large proportion of the corm covered by the lesions, by confluence of the lesions, and often by the presence of secondary organisms.

[1] Massey, L. M.   The hard rot disease of gladiolus.   N. Y. (Cornell) Agr. Exp. Sta. Bul. 380: 149–181.   1916.

[2] Pape, H.   Die Hartfäule-Krankheit der Gladiolen und ihre Bekämpfung.   Die Gartenwelt 29 (40) : 670–680.   1925.

[3] Moore, W. C.   Diseases of bulbs.   [Gt. Brit.] Min. Agr. and Fisheries.   Bul. 117. 1939.

NUTRITION OF THE TREES AND DEVELOPMENT OF DUTCH ELM DISEASE

K. G. PARKER, L. J. TYLER, D. S. WELCH,
AND SETH POPE[1]

Observations in the field on the Dutch elm disease have indicated that elm trees in high, moderate, and low vigor are susceptible to the disease (3). Where differences in susceptibility have been reported the observations were that rapidly growing trees were somewhat more severely diseased than were slowly growing ones. Buisman (2) observed this difference where part of the trees were induced to grow more rapidly by pruning.

Considerable importance is attached to keeping elm trees in reasonably good condition in order to prevent the breeding of bark beetles. This, of course, would help in keeping down the population of the beetles. More important is the prevention of inoculations with *Ceratostomella ulmi* (Schwarz) Buis. by bark beetles attracted to the trees, or by the progeny of these beetles. The question immediately arises as to whether the maintenance of a tree in condition to prevent beetle breeding might result in its being more likely to be destructively affected by the disease if it should be inoculated.

Went (7) obtained less development of the Dutch elm disease on potted trees given a full nutrient treatment and supplied with extra potassium than on those given the full nutrient treatment only. Banfield (1) grew trees in sand and supplied a full nutrient solution. Less dieback resulted on these than on trees treated similarly but with nitrogen withheld. On the other hand, if he pruned the trees heavily, with a resultant very long and succulent shoot growth, those which received nitrogen were more severely diseased than were those which did not. May (4) attributed the different responses in these trees to different carbohydrate-nitrogen ratios. Zentmyer and Wallace (8) stated that trees that responded well to nitrogen fertilization were less severely affected than were others.

MATERIALS AND METHODS

Trees of the species *Ulmus americana* L. were used in all experiments. In experiment 1 they were 2-year-old budded trees of the Princeton variety. For the other two experiments, cuttings were rooted from a clonal selection made by the Jackson and Perkins Nursery, Newark, New York. The trees

used in the last two experiments were grown from cuttings rooted in 1936, with the experiments started in 1938 and in 1939, respectively.

The trees were potted in 18-quart galvanized pails in a very poor Gloucester loam soil, about 17 kilograms per tree. This soil was so poor that not more than traces of nitrogen, phosphorus, and potassium could be detected by the "quick test" methods of analysis used in this study. The pH of the soil when collected from the field usually was about 5 to 5.5. Subsoil was used in experiment 1 and topsoil in the other two experiments.

At the time of planting any phosphorus or potassium materials to be added were mixed in the soil for each tree before it was potted. Nitrogen compounds were stirred into the top two inches of soil after potting. Where the entire complement of nitrogen was to be supplied in the form of a chemical compound, only one-half of the amount was applied at potting. The remainder was added three to four weeks later. The entire complement of potassium or phosphorus was applied in the initial treatment. Where manure was used the entire treatment was made at potting, including all of the nitrogen.

The pails were sunk into the ground to the depth of the soil inside and were spaced in rows so that the trees were 18 or 24 inches apart, depending on their size.

After the first year in any given experiment treatments were made similarly to those at the outset. The first treatment was made about the time growth started in the spring and the materials were stirred into the top three to four inches of soil. The second application of nitrogen was made three to four weeks later.

During the summer months water was applied as needed. The soil was stirred every week or two to help maintain it in good tilth.

The trees were pruned at planting and in the spring one year later. Those in experiments 1 and 2 were pruned very moderately and those in experiment 3 somewhat more heavily. No pruning was done after the inoculations were made. Toward the end of each growing season, and at the termination of the experiment, soil was collected from experiments 2 and 3 for analysis and pH determination. The top one-half inch of soil was removed and a hole about four inches deep dug with a trowel. The sample was taken by scraping with the trowel along the sides of the hole, taking care to obtain soil from top to bottom. Each test sample was a composite of two taken from opposite sides of a single tree. At the termination of the experiment all of the soil was removed and a second sample taken from the entire mixed soil contents of the individual pail. Tests were made at once on the fresh soil; or it was spread out to dry, then stored in paper bags in an office, away from any laboratory fumes, until tests could be made.

Hydrogen-ion determinations were made by means of a Beckman pH meter, model G, using the 5-inch glass electrodes designed especially for work with heavy suspensions. The soil was mixed with distilled water in the ratio of 1 to 5 or 1 to 2.5, and the suspension was stirred vigorously for one minute with the electrodes in it before the reading was taken.

Analyses for nutrients were made by means of the quick tests developed by Morgan (5). Morgan described a modification of these tests in a later publication, but his earlier methods were followed throughout for uniformity.

Relative growth rates sometimes were determined by measuring the total length of all current season's shoots on each tree. In part of the experiments the fresh weight of each tree was taken at the beginning and at the end. Also, the thickness of the annual rings in the wood was measured at the time the trees were cut for examination.

In the second year of each experiment, the trees were inoculated by the method described by Tyler (6). The spore suspension of *Ceratostomella ulmi* was injected on opposite sides of the trunk just below the first branch. This was done after considerable growth had been produced but while shoot length was being extended, usually about June 1. Examinations for wilt and defoliation were made at various intervals during the summer, depending on the rate of development of symptoms.

At the end of the second growing season, the year in which the trees were inoculated, the trees in experiment 1 were removed from the pails, the soil removed from the roots, and various examinations made for severity of disease development on each tree. The trees in experiment 2 were not examined until the spring two years after they were inoculated and those in experiment 3 were examined in October, one and one-half years after they were inoculated.

The extent of invasion by the pathogen was determined by measuring the distance disease discoloration extended from the inoculation point. Also, the total length of the trunk and main branches and the portion of each that had been killed were measured.

Sufficient cultures were prepared to make sure that the trees were affected by the Dutch elm disease and that the discoloration present was caused by that disease.

Experiment 1 was designed to test the influence of an inorganic fertilizer treatment containing nitrogen, phosphorus, and potassium singly, and in all possible combinations, on the development of the disease. Nitrogen was supplied in the form of sodium nitrate, phosphorus as superphosphate, and potassium as potassium sulphate. The complete treatment contained these elements in the ratio of 5–10–5, nitrogen expressed as N, phosphorus as P_2O_5, and potassium as K_2O. In the second year, only the nitrogen was supplied, since spectroscopic analyses[2] made on soil from several of the trees indicated phosphorus and potassium were present in abundance. The nitrogen application was reduced by one-half that year because some nitrogen injury to the leaves had appeared the first season.

The treatments were as listed in table 1.

The trees were potted on April 22 and 23, 1935, and the experiment was terminated in October, 1936.

Since the results of experiment 1 showed a definite need for a complete

TABLE 1.—*Formulae and materials applied to trees in experiment 1 the first year*

Treatment	Formulae	Materials, in grams per tree		
		Sodium nitrate	Superphosphate (16 per cent P$_2$O$_5$)	Potassium sulphate
1 (check)	0–0–0	0	0	0
2 (NPK)	5–10–5	56	112	18
3 (K)	0–0–5	0	0	18
4 (N)	5–0–0	56	0	0
5 (P)	0–10–0	0	112	0
6 (PK)	0–10–5	0	112	18
7 (NK)	5–0–5	56	0	18
8 (NP)	5–10–0	56	112	0

fertilizer treatment, experiment 2 was designed to test different formulae (Table 2). Also, the effects of different hydrogn-ion concentrations in the soil and the addition of minor elements were studied in this experiment.

The trees were planted in April, 1938. Since analyses made in October, 1938, indicated only moderate amounts of available nutrients left in the soil, the same formulae were used in 1939 as in the first year, with the following exceptions: No lime was applied to trees in treatment 5. The amounts of N, P$_2$O$_5$, and K$_2$O supplied to all trees were increased to 1½ times that used in 1938 because of the greater size of the trees.

In 1940 the soil treatments were the same as those made in 1939 except that no minor elements were added to the trees in treatment 6.

The trees were cut and results recorded on May 13 to 16, 1941. No soil treatments were made in 1941.

TABLE 2.—*Formulae and materials applied to trees in experiment 2 the first year*

Treatment	Formulae	Materials, in grams per tree		
		Sodium nitrate	Superphosphate (20 per cent P$_2$O$_5$)	Potassium sulphate
1 (check)	0–0–0	0	0	0.0
2	5–10–5	20	32	6.4
3	10–10–5	40	32	6.4
4	6–8–3	24	25.6	3.8
5	5–10–5 (plus lime)[a]	20	32	6.4
6	5–10–5 (plus minor elements)[b]	20	32	6.4

[a] Forty-five grams ground limestone per tree added.
[b] Minor elements were added in solution, per tree as follows:

Compound	Number of grams	Actual amount of element (gm.)
CuSO$_4$ · 5H$_2$O	0.1936	0.048 Cu
MnCl$_2$ · 4H$_2$O	0.384	0.096 Mn
Na$_2$B$_4$O$_7$ · 10H$_2$O	0.03	0.0036 B
FeSO$_4$ · 7H$_2$O	0.432	0.096 Fe
KI	0.0624	0.048 I

Experiment 3 was designed to test 3 different nitrogen sources, different formulae, and two levels of treatment in a complete fertilizer. The experiment was started in 1939 and the treatments in 1940 and 1941 were the same as those in 1939. The formulae used are given in table 3. It was necessary

TABLE 3.—*Formulae and materials applied to trees in experiment 3 the first year*

Treatment number	Formulae	Materials, in grams per tree				
		Urea (42 per cent N)	Sodium nitrate	Super-phosphate (20 per cent)	Potassium sulphate	Manure[a]
1 (check)	0–0–0	0.0	0	0	0.0	0
2	5–10–5	0.0	25	40	8.0	0
3	5–10–5	9.5	0	40	8.0	0
4	6–8–3	11.4	0	32	4.8	0
5	5–10–5	4.7	0	36	4.2	110
6	2 × (5–10–5)	19.0	0	80	16.0	0
7	2 × (6–8–3)	22.8	0	64	9.6	0

[a] Containing: N—1.85 per cent; P_2O_5—0.75 per cent; K_2O—1.75 per cent.

to change the amounts of materials applied in treatment 5 after the first year to compensate for a slightly different analysis of the manure used.

Final results on the trees in experiment 3 were recorded in October, 1941.

RESULTS OF EXPERIMENTS

Experiment 1

During the first year the best growth, as judged by their general appearance, occurred in the trees which received treatment 2, the complete fertilizer (Table 4). Next best was treatment 8, nitrogen and phosphorus. There were some leaves with brown edges in all treatments, including the check. A part of this injury probably was caused by the fertilizers; but it was not possible to assign the injury to particular elements, or to assess its severity. Again in 1939 treatment 2 was best, with shoot lengths in treatment 7 good but color of leaves becoming poor during the summer. Treatment 8 was next to treatment 2. All others were poor; however, there was no definite fertilizer injury evident in any treatment.

In experiment 1 the least wilt and the least extensive invasion of the trees occurred in the trees which received the complete fertilizer. Next in order was the treatment of nitrogen and phosphorus, and third was the nitrogen-potassium combination. Other treatments were little, if any, better than the nonfertilized control. These results were correlated inversely with the amount of growth, the greater the growth the less was the disease development.

Experiment 2

Growth on the trees was uniformly good except on the nonfertilized controls (Table 5). Fertilizer injury to the leaves became evident by August, 1939. It was most severe on treatments 3, 4, and 6, moderate on 2, and least

on 5.   There was no clear indication of relationship with the different materials applied with the possible exception that lime may have prevented the injury to some extent.   Likewise, growth was somewhat greater on the trees that received lime.

The pH of the soil in August, 1938, ranged between 5.8 and 6.0 in all treatments except that including lime, in which it was 6.6.   At the end of the experiment similar relationships held, but the hydrogen-ion concentration in the sample taken from the composite sample of all soil in the pail was markedly higher than that in the surface soil sample.   All nutrients usually fell in the medium range in the treated soils and in the low range in the nonfertilized controls.

TABLE 4.—*Severity of disease development and growth of trees in experiment 1. Each figure is the average from 6 trees, inoculated June 3, 1936*

Treatment	Thickness of annual rings		Leaves affected[a]		Discoloration[d]	Dead wood in main branches[a]
	1935	1936	Greatest amount observed[b]	Observed Aug. 17, 1936[c]		
	Mm.	*Mm.*	*Per cent*	*Per cent*	*Inches*	*Per cent*
1 (check)	0.5	0.4	85.8	75.8	29.2	22.5
2 (NPK)	0.7	1.5	15.8	9.2	4.3	3.3
3 (K)	0.6	0.3	89.2	68.3	21.7	30.8
4 (N)	0.5	0.4	82.5	65.0	20.4	22.2
5 (P)	0.4	0.5	90.0	85.8	27.1	8.3
6 (PK)	0.4	0.5	71.7	60.8	18.6	22.2
7 (NK)	0.4	0.7	58.2	31.2	17.8	5.0
8 (NP)	0.5	1.0	47.5	21.3	14.6	0.3

[a] Estimated, expressed as percentage.
[b] Amount of leaves wilted varied from time to time and recovery was definitely observed.   Hence greatest amount wilted during the summer on most trees was greater than that on August 17.
[c] Last date when observations on wilt were made.
[d] The discoloration extended downward from the point of inoculation.

In order to save space in table 5 data on weight of the trees are not given. Differences in weight corresponded very closely with those in total length of the 1938 shoot growth.

Based on amount of wood killed, the least disease development occurred in treatment 3, with the 10–10–5 fertilizer formula.   There was little to choose from, however, among the treatments except that the trees receiving lime were more severely affected.   All treatments were much better than the check.

Experiment 3

As in the previous experiment, growth of the trees in this test was generally good except in the nonfertilized control group (Table 6).   No injury attributed to the fertilizer treatment was noted at any time.

The pH of the soil in this experiment was generally similar to that in experiment 2.   Where part of the nutrients were supplied in the form of

TABLE 5.—Measurements of growth and disease development in experiment 2. Trees inoculated May 29, 1939

Treatment number	Fertilizer formulae	Total length 1938 shoots, av. per tree in inches	Wilt in 1939, av. per tree [c]		Condition of wood [e] in May, 1941					
			Greatest amount observed	Observed July 5 [d]	Trunk			Main branches		
					Total inches	Dead wood		Total inches	Dead wood	
						Inches	Per cent		Inches	Per cent
1[a]	Check	50	86.7	78.3	237	116	48.9	287	247	86.1
2[a]	5-10-5	180	30.8	26.8	260	16	6.2	809	172	21.3
3[b]	10-10-5	176	38.5	27.0	197	0	0.0	568	71	12.5
4	6-8-3	173	30.0	18.7	265	22	8.3	697	141	20.2
5[a]	5-10-5 (plus lime)	190	50.4	34.4	288	21	7.3	608	201	33.1
6[a]	5-10-5 (plus minor elements)	147	34.8	28.9	252	10	4.0	584	98	16.8

[a] 12 trees in treatment.
[b] 10 trees in treatment.
[c] Estimated percentages.
[d] Observations at later dates not reliable because of fertilizer injury.
[e] Data given are sums of measurements of all trees.

TABLE 6.—*Measurements of growth and disease development in experiment 3.[a] Trees inoculated May 30, 1940*

Treatment number	Fertilizer formulae	Total length 1939 shoots, av. per tree in inches	Wilt in 1940, av. per tree		Condition of wood[f] in October, 1941					
			Greatest amount observed	Observed July 31	Trunk			Main branches		
					Total inches	Dead wood		Total inches	Dead wood	
						Inches	Per cent		Inches	Per cent
1	Check	65	98	92	211	105	49.8	264	256	97.0
2	5-10-5[b]	138	93	85	194	43	22.2	314	225	71.7
3	5-10-5[c]	200	86	79	227	43	18.9	301	220	73.1
4	6-8-3[e]	194	80	74	214	70[g]	32.7	339	238	70.2
5	5-10-5[d]	177	81	79	208	41	19.7	361	205	56.8
6	2 × (5-10-5)[c]	229	94	86	197	104[h]	52.8	374	316	84.5
7	2 × (6-8-3)[c]	188	89	85	201	90[g]	44.8	340	259	76.2

[a] 10 trees in each treatment.
[b] Nitrogen source was sodium nitrate.
[c] Nitrogen source was urea.
[d] Dried cow manure included, with remainder of nitrogen supplied as urea.
[e] Estimated percentages.
[f] Data given are sums of measurements of all trees.
[g] Two trees entirely dead.
[h] Four trees entirely dead.

cow manure the hydrogen-ion concentration was lower than it was in the other treatments, particularly at the end of the experiment. In the non-fertilized controls the hydrogen-ion concentration at the end of the experiment was markedly higher than in the treated soils.

Nutrients in the treated soils were generally in the high range throughout, and in the nonfertilized controls they were always low.

There was more dead wood in the trees which received the 6–8–3 fertilizer formula than the 5–10–5. Two trees· which received the 6–8–3 treatment were completely killed and two were killed in the $2 \times (6–8–3)$ treatment. Four trees which received the double amount of 5–10–5 were killed (treatment 6). It seemed to make little difference in this experiment whether the nitrogen was supplied in the form of sodium nitrate, urea, or a mixture of urea and barnyard manure. All (applied in the 5–10–5 formula, smaller amount) were equally good, much better than the check, and appreciably better than the 6–8–3 formula or the double dosage of the 5–10–5 formula.

More severe disease development occurred generally in this experiment than with comparable soil treatments in experiment 2, probably because of the heavier pruning these trees received. These results are in agreement with those of Banfield (1) and of Buisman (2).

<div align="center">DISCUSSION</div>

It seems fairly clear that elm trees may be maintained in good growing condition without inducing unusually severe development of the Dutch elm disease in inoculated individuals. The indications are that soil treatments should not be excessive and should be well balanced. However, specific fertilizer formulae cannot be devised on the basis of the information available. Pruning of the trees should be kept at a minimum.

Nitrogen obviously is necessary but care should be exercised in the amounts applied. Of particular significance is the apparent need for phosphorus. These experiments indicate a very definite benefit from phosphorus in aiding the tree to survive when diseased. Potassium appears to be of less importance but was beneficial in a treatment for the very poor soil used in this study.

Experiments were made on larger trees planted in the open, with the results similar but the differences not so large. That is, growth differences were less, as were differences in disease development. However, when a good response to a complete fertilizer treatment was obtained, the trees were less severely affected by the Dutch elm disease than were the nonfertilized and more poorly growing check trees.

Therefore, there appears to be no danger in keeping trees in a good growing condition so long as they are not pruned too heavily and a succulent type of tissue is not produced. Probably a complete fertilizer, not very high in nitrogen, should be used until further investigation has determined more accurately the requirements of a good treatment.

In one experiment the hydrogen-ion concentration was reduced by the

addition of lime to the soil, and greater disease development resulted. In other tests the hydrogen-ion concentration in the soil was less in the better treatments than in the checks. Obviously, the influence of pH of the soil on the development of this disease cannot be determined on the basis of these experiments, but it appears to deserve further study.

SUMMARY

Experiments with small trees potted in subsoil indicated that a complete fertilizer treatment may result in less severe disease development in trees infected by the Dutch elm disease pathogen. Nitrogen and phosphorus seemed to play a major rôle, with potassium in a secondary position but, nevertheless, important.

Among the various nitrogen sources tried, in experiments using topsoil, there were no definite differences.

Excessive amounts of fertilizer, over that required to support good growth, may be undesirable.

Pruning sufficiently heavily to induce very succulent shoots resulted in increased disease development.

There was some indication that differences in disease development may result, directly or indirectly, from differences in hydrogen-ion concentration in the soil.

CORNELL UNIVERSITY,
ITHACA, NEW YORK.

LITERATURE CITED

1. BANFIELD, W. M. Effect of nitrogen on inception and development of Dutch elm disease on American (Moline) elm. (Abstr.) Phytopath. **28**: 2–3. 1938.
2. BUISMAN, CHRISTINE. Verslag over de onderzoekingen betreffende de iepenziekte, verricht in het phytopathologisch laboratorium "Willie Commelin Scholten" to Baarn, gedurende 1934. Tijdschr. over Plantenz. **41**: 104–120. 1935.
3. MAY, CURTIS. Outbreaks of the Dutch elm disease in the United States. U. S. Dept. Agr. Circ. 322. 1934.
4. —————. Host-parasite relations of *Ceratostomella ulmi*. (Abstr.) Proc. Third Int'l. Cong. for Microbiol. 1939: 530–531. 1940.
5. MORGAN, M. F. The Universal soil testing system. Connecticut Agr. Exp. Sta. Bul. 392: 129–159. 1937.
6. TYLER, L. J. Influence of temperature on the Dutch elm disease in potted American elm. Phytopath. **35**: 302–304. 1945.
7. WENT, JOHANNA C. Verslag van de onderzoekingen betreffende de iepenziekte, verricht op het phytopathologisch laboratorium "Willie Commelin Scholten" te Baarn, gedurende 1936. Tijdschr. over Plantenz. **43**: 75–90. 1937.
8. ZENTMYER, GEORGE A., and PHILLIP P. WALLACE. Factors influencing the development of *Ceratostomella ulmi* in elm trees. (Abstr.) Phytopath. **34**: 1014. 1944.

BACTERIAL LENTICEL INFECTION OF EARLY POTATOES

M . A . SMITH[1] AND G . B . RAMSEY[2]

Many of the previous extensive investigations on bacterial soft rot of potatoes have been concerned chiefly with tubers showing decay following mechanical injuries or heat injury (scald) during harvesting, transit, and storage (3, 11, 12, 13, 14, 15, 16, 17). While bacterial lenticel infection of potatoes has been reported (13, 18, 19), no critical study has been made of the types of injury and decay resulting from such infections, the conditions under which such infections occur, or the identity of the organisms involved. This paper is the result of studies since 1942 of bacterial lenticel infection of early potatoes and is concerned primarily with the pathogenicity of isolates from lenticels and other sources, the pathological histology of the host, and the physiological characteristics of the organisms involved.

Observations of early potato shipments arriving on the Chicago market since 1935 reveal that bacterial infection of potatoes through lenticels has been an important factor in the incidence of bacterial soft rot (13), particularly since the washing of potatoes has become common practice.

Cars of potatoes arriving on the Chicago Produce Terminal in such poor condition as to require reconditioning before they can be sold, are shifted to a special track and unloading platform. A careful check of many of these cars during the past 4 years shows that bacterial soft rot is the most important cause of decay. Some carlots have so much decay as to make necessary the sorting of potatoes in all bags. In many cars, however, most of the bags with excessive decay are in the warmest and poorest ventilated part of the load, halfway between doorway and bunkers at each end of the car. Shipping tests have shown that this part of the load is most likely to decay (14).

In 1942 a survey of 132 carlots of potatoes reconditioned in Chicago showed that loss on account of bacterial soft rot ranged from 2 to 98 per cent. Losses of 1000 to 10,000 lb. were common, and in many lots 15,000 to 24,000 lb. out of a 30,000-lb. load were worthless. Most of these shipments originated in States having excessively wet weather before and during harvesting, and most of the decay that followed originated at lenticels. Table 1 shows the percentage of decay found on reconditioning some lots of early potatoes from various States.

Decay of potatoes following lenticel invasion by bacteria is usually more serious in stock grown in wet or heavy soils than in light soils. Soft-rot bacteria have been isolated from lenticels that appeared normal as well as from enlarged and proliferated lenticels. The universal presence of soft-

rot bacteria in soil makes possible the invasion of lenticels previous to harvesting while subsequent temperature, moisture, and handling conditions determine whether or not decay eventually develops. As is well known, tubers grown in heavy or wet soils often have greatly proliferated lenticels and bacterial invasion often occurs in this proliferated tissue (Fig. 1, B). When these tubers are washed before shipment, the proliferated tissue is often rubbed off and the injured areas resulting are readily invaded by bacteria present in the wash water. Infected lenticels may remain as mere blemishes but bacterial soft rot often develops at such centers under favorable conditions. Warm potatoes, bagged wet, and loaded into non-refrigerated cars are under conditions most favorable for the development of extensive bacterial soft rot (14).

Lenticel infection is often characterized by raised, watersoaked areas, 3 to 5 mm. in diameter, around the lenticels (Fig. 1, A). The tissue in such

TABLE 1.—*Bacterial soft rot following lenticel infections and injuries in some typical lots of potatoes reconditioned on the Chicago Market in 1943*

Origin	No. bags	Date	Percentage of decay	
			Range	Average
Alabama	3955	May–June	5–50	14.6
Arkansas	526	June–July	10–21	15.2
California	3257	May–June	3–28	11.1
Kansas	2251	June–July	5–72	34.2
Louisiana	4592	May–June	2–70	13.9
Missouri	5865	June–July	7–93	46.5
Nebraska	2739	July–August	2–26	11.9
Oklahoma	1414	June–July	6–98	24.6

areas is usually firm. The swelling and watersoaking of the infected area is commonly observed in the varieties Bliss Triumph and Warba, and occasionally in Cobbler. When cross sections are made of infected areas, the tissues beneath appear watersoaked and gray to dark brown (Fig. 2, D). The depth of the infected area varies from 1 to 3 mm. Although lenticels of White Rose and Cobbler are often infected without showing evidence of swelling, tubers of both these varieties with swollen, infected lenticels are occasionally seen.

When drying occurs in the infected area around the lenticels the epidermis often becomes slightly sunken, although it generally remains intact (Fig. 1, C, D). Cross sections through the infected areas of such lenticels reveal that the outer periderm and the infected parenchyma cells beneath have become separated, resulting in the formation of a small cavity between them (Fig. 2, F). In the varieties Cobbler and White Rose the color of the epidermis over the lesions varies from gray to tan with a darker border. In the red-skinned varieties, such as Warba and Triumph, the infected area often appears bleached. Lenticel infections are often so numerous that they coalesce making individual spots indistinguishable (Fig. 1, D). Bacterial invasion of proliferated lenticels often appears as watersoaked, light
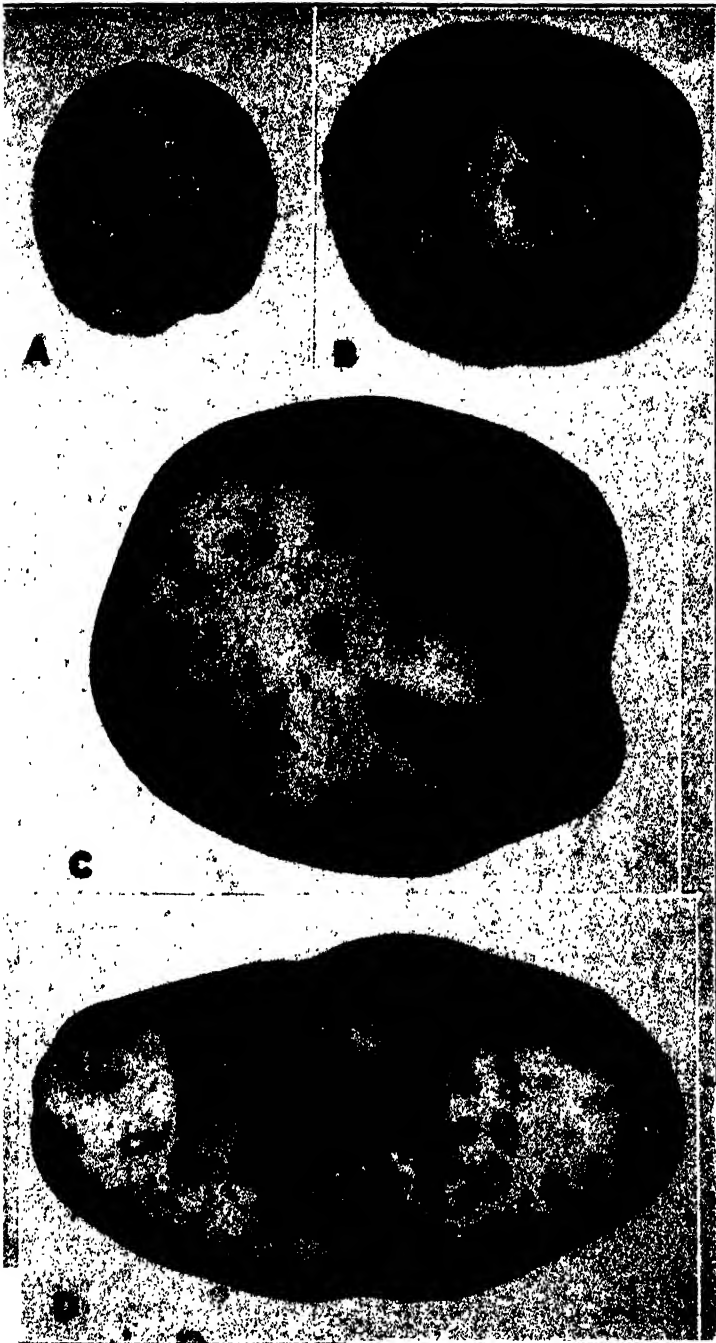
FIG. 1.   A. Bacterial lenticel infection on Bliss Triumph potato tuber showing swollen areas around lenticels.   B. Decay around enlarged lenticels on Bliss Triumph. C. Various stages of decay at lenticels on Cobbler.   D. Lenticel infection on White Rose. Some lesions have coalesced resulting in advanced decay.

brown areas in the tissues in the immediate vicinity although it may extend
2 to 3 mm. around the lenticels (Fig. 1, B). The diseased area may extend
into the tuber to a depth of 2 to 4 mm.

PATHOGENICITY

The bacterial isolates used in these studies were obtained from represen-
tative varieties of potatoes from the principal early potato producing sec-
tions of the United States. Isolations were made from lenticels, soil around
tubers, tubers in advanced stages of soft rot, tubers in which rot was around
mechanical injuries, and from tubers that were browned and had become
sticky because of superficial bacterial growth in areas where the epidermis
had been rubbed off. In the studies of infected lenticels, isolations were
made from those having swollen, watersoaked areas around them (Fig. 1, A),
sunken, decayed areas around lenticels (Fig. 1, C, D), and decay around
proliferated lenticels (Fig. 1, B). Isolations from potatoes with advanced
soft rot were made from the interior of tubers along the margin of the
farthest advance of the decay.

The various isolates were tested for pathogenicity by needle puncture
into potato slices or whole tubers. In the inoculation of lenticels by artifi-
cial means, noninjured tubers were allowed to remain in water suspensions
of bacteria for various lengths of time, after which they were incubated in
tightly sealed moist chambers.

The summary of the pathogenicity studies appears in table 2. With the
exception of isolates from skinned, browned tubers that had become sticky,
a relatively high percentage of the isolates from the different sources were
pathogenic. The average percentages of pathogenic isolates from the dif-
ferent sources were as follows: Soil around tubers, 78.4; swollen, water-
soaked areas around lenticels, 74.0; decay around proliferated lenticels,
71.9; sunken, decayed areas around lenticels, 60.0; tubers with advanced
soft rot, 66.3; decay around mechanical injuries, 52.3; and sticky areas on
browned tubers, 0.

During the studies, observations were made to determine if there were
marked pathogenic differences among the various isolates. Cultures were
used from isolation series 2, 4, 5, 6, 9, 10, 14, 16, 21, 22, 23, 27, and 30.
This series included isolates from all of the different sources. The rapidity
and extent of the rot in whole tubers was considered a measure of patho-
genicity. The results of the tests indicated that there was much variation
in the progress and extent of rot among isolates from different series and
even among isolates from the same series. Some were strongly pathogenic,
some moderately so, and others weakly pathogenic. Leach (10) found in
studies with blackleg of potato that it was almost impossible to rot tubers
with the blackleg pathogen by wound inoculations when tubers were given
a constant supply of fresh air even though inoculated at the optimum tem-
perature and in a saturated atmosphere. In the present studies soft rot
readily occurred when inoculated tubers were placed in tin pails with

tightly fitting tops or in moist chambers of the desiccator type where the oxygen supply was not replenished.

Certain of the isolates that caused rapid rotting (Series 2, 6, 10, 16, 22, and 30) produced an extensive, soft, grayish-white decay of tubers. Isolates from other series induced a decay that varied from white to dark brown.

TABLE 2.—*Pathogenicity to potato of bacterial isolates from different sources*

Isolation series No.	Location and host variety	Isolates		
		Number	Source	Percentage pathogenic
1	Missouri—Warba	13	Swollen, watersoaked areas around lenticels	69.2
2	Do	19	Soil around tubers	73.6
3	Missouri—Cobbler	12	Do	83.3
4	Do	14	Decay around proliferated lenticels	64.2
5	Do	9	Tubers with advanced soft rot	66.6
6	Nebraska—Warba	6	Sunken, decayed areas around non-proliferated lenticels	50.0
7	Do	7	Swollen, watersoaked areas around lenticels	71.4
8	Do	18	Sticky areas on browned tubers	0
9	Do	12	Tubers with advanced soft rot	75.0
10	Nebraska—Cobbler	4	Swollen, watersoaked areas around lenticels	75.0
11	Florida—Triumph	16	Do	50.0
12	Kansas—Cobbler	8	Sunken, decayed areas around non-proliferated lenticels	62.5
13	Do	9	Decay around proliferated lenticels	77.7
14	Do	10	Decay around shatter bruises	50.0
15	Louisiana—Triumph	10	Swollen, watersoaked areas around lenticels	100.0
16	Do	6	Sunken, decayed areas around non-proliferated lenticels	66.6
17	Do	15	Decay around proliferated lenticels	73.3
18	Do	20	Tubers showing advanced soft rot	70.0
19	Texas—Triumph	18	Swollen, watersoaked areas around lenticels	66.6
20	Do	7	Sticky areas on browned tubers	0
21	Alabama—Triumph	12	Swollen, watersoaked areas around lenticels	91.6
22	Do	9	Sunken, decayed areas around non-proliferated lenticels	66.6
23	Do	16	Decay around shatter bruises	50.0
24	Do	13	Tubers showing advanced soft rot	53.8
25	Colorado—Cobbler	16	Swollen, watersoaked areas around lenticels	75.0
26	Do	17	Sunken, decayed areas around non-proliferated lenticels	64.1
27	Washington—White Rose	11	Do	63.6
28	Do[4]	10	Swollen, watersoaked areas around lenticels	70.0
29	California—White Rose	20	Sunken, decayed areas around non-proliferated lenticels	50.0
30	N. Dakota—Triumph	14	Decay around cuts	57.1

None of the isolates from skinned tubers with sticky browned areas proved pathogenic. A study of certain of these nonpathogenic organisms revealed that they resembled *Bacterium coadunatum* (Wright) Bergey *et al.* (4) in certain morphological, biochemical, and physiological characteristics.

This organism was found by Brooks and McColloch (5) to be capable of producing stickiness in shelled green lima beans. It has been observed that under favorable conditions, such as high relative humidity and moderate temperature, bacterial soft rot may follow infection by some of the sticky
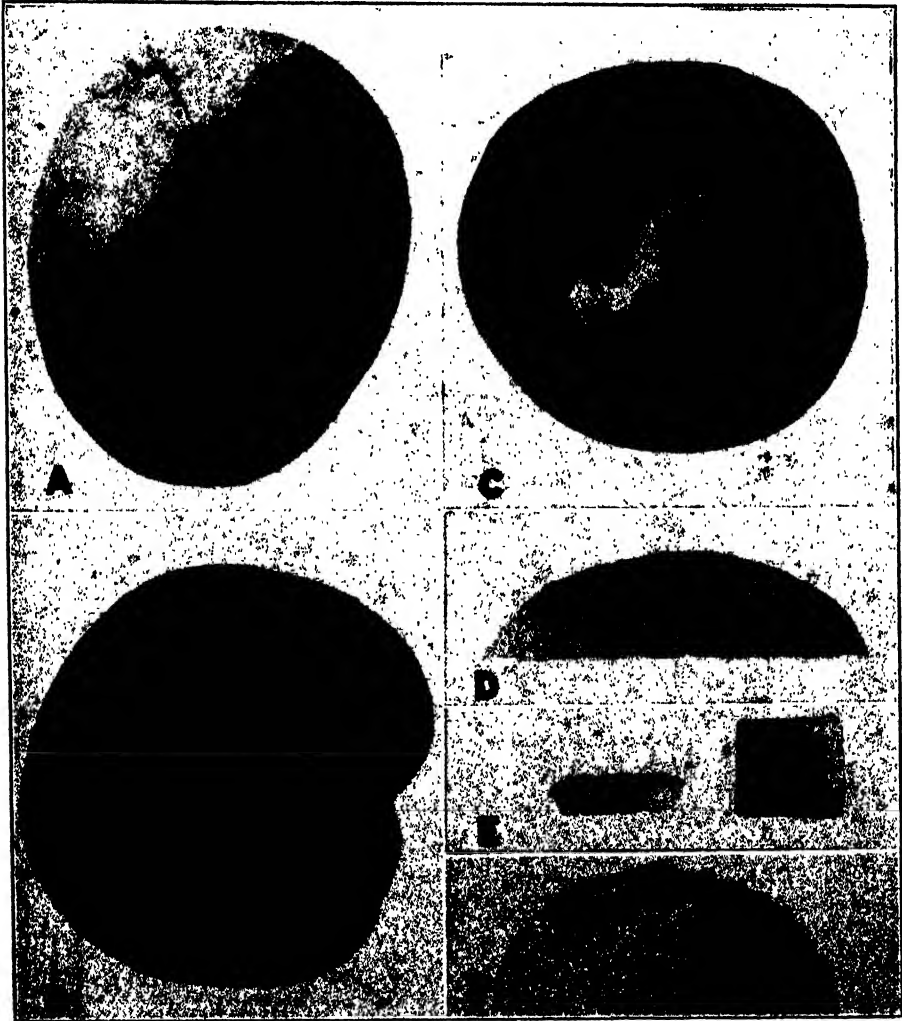
FIG. 2. A. Infected lenticels 24 hr. after artificial inoculation. B. Decay followed by collapse of tissue in infected area; 48 hr. after inoculation. C. Lenticel infection of Bliss Triumph 48 hr. after inoculation. D. Cross section through infected area (from C) to show depth of decay. E. Cross section through artificially inoculated lenticel (at left). Sunken, infected area around lenticel (at right). F. Section through naturally infected lenticel on White Rose. Periderm has become separated from underlying tissue.

organisms. However, evidence of this secondary infection has been observed only rarely in shipments of early potatoes under ordinary transit conditions.

Smith (19) was one of the first investigators to recognize the lenticels of the potato as avenues of infection for bacterial organisms.   He reported first observing bacterial lenticel infection of potatoes in 1886.   Later, using *Bacillus melanogenes* Pethybr. and Murphy (which he stated was a mixture of *Bacillus phytophthorus* Appel and *Bacillus solanisaprus* Harrison) he obtained typical infection of tubers by way of the lenticels.   Leach (9) has considered *B. phytophthorus* and *B. solanisaprus* to be strains of *Erwinia carotovora*.

In the present studies, an investigation of certain phases of lenticel infection with pure cultures of lenticel isolates seemed desirable.   In a preliminary experiment, 10 recently harvested Bliss Triumph potatoes were washed in soap and water, rinsed in distilled water, dipped in 80 per cent alcohol and allowed to dry in the air.   An 18-hour-old agar slant culture from series 6 (Table 2) was added to distilled water and 5 of the tubers were immersed in this suspension where they were allowed to remain for 24 hours, after which they were removed and placed in moist chambers where a saturated atmosphere was maintained.   The temperature during the incubation period was 78° F.   For controls, the remaining 5 tubers were immersed in distilled water for 24 hr. after which they were removed and placed in moist chambers.   Infections centering at lenticels were first apparent on some of the tubers 18 hr. after removal from the inoculum and lenticel infections appeared on all of the tubers within 24 hr.   The infection was often characterized by dark, brown, watersoaked areas 1 to 3 mm. in diameter around the lenticels (Fig. 2, C).   The infected areas were often swollen (Fig. 2, A), but on some tubers the tissues in the infected areas were slightly sunken (Fig. 2, B).   When infected lenticels were examined, large numbers of bacteria could be seen in them and the organism was readily reisolated in pure culture.   Control tubers showed no evidence of bacterial lenticel invasion during the 24-hr. period.

Another experiment, following the technique described, was conducted with the varieties Cobbler and White Rose and a lenticel isolate from series 12.   Of the 12 inoculated tubers, 4 of the Cobbler and 3 of the White Rose bore a number of lenticel infections 18 hr. after inoculation and all of the tubers of both varieties had numerous infected lenticels 24 hr. after inoculation.   Control tubers remained free of infection during the course of the experiment.

An experiment using similar inoculation methods was carried on in an attempt to determine the length of immersion time necessary to obtain lenticel infections.   Twenty-four recently harvested Bliss Triumph potatoes were washed in distilled water, immersed in 80 per cent alcohol for 1 minute, and allowed to dry in the air.   These tubers were divided into 6 lots of 4 each and the first 5 lots were then immersed in a water suspension of bacteria from an 18-hr.-old culture of an isolate from series 16 for 1, 2, 3, 4, and 5 min., respectively.   The sixth lot of 4 tubers was immersed for 5 min. in the distilled water that had been used in washing the tubers at the begin-

ning of the experiment.   Controls consisted of 6 lots of 4 tubers each which were washed, disinfected, and then immersed in distilled water for the various times mentioned.   At the termination of each period the tubers were removed from the inoculum and placed in moist chambers in which the atmosphere was near saturation.   The temperature during the experiment was 72° F.   Final observations were made after an incubation period of 48 hr.   The experiment was repeated after an interval of 2 days with an isolate from series 21.   The results appear in table 3.

TABLE 3.—*Relationship of time to infection of potatoes by immersion inoculation with lenticel isolates*

Inoc. time (min.)	Av. no. infected lenticels per tuber[a]	Av. diameter of infected area around lenticels (mm.)
Expt. 1. Isolate from Series 16.		
1	7	4.0
2	9	4.7
3	7	3.9
4	10	6.5[b]
5	7	5.0
5 (wash water)	8	6.8
Controls		
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
5	0	0
Expt. 2. Isolate from Series 21.		
1	3	3.8
2	4	4.1
3	7	4.7
4	11	6.0
5	6	5.4
5 (wash water)	4	5.9[b]
Controls		
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
5	0	0

[a] In each instance 4 tubers had been inoculated.
[b] Infected areas coalesced.

Lenticel infections were obtained on tubers immersed in the inoculum for 1, 2, 3, 4, and 5 min., respectively.   In these experiments the average number of infected lenticels was as large (or larger) when tubers were immersed in the inoculum for 1 and for 2 min. as when they were immersed for 5 min., thus indicating that prolonging the immersion time had little effect on the number of infections.

In commercial washing practice, tubers often remain in the wash water from 1 to 2 min.   This water may be expected to contain large numbers

of bacteria, some pathogenic to potato. If tubers have not already become contaminated with bacterial organisms, there will be ample opportunity for this to occur in the washing process. Frequent changing of wash water or the use of fresh water sprays has been recommended as an aid in cutting down the bacterial content of the wash water. In this connection, however, it is of interest to note the results obtained when tubers were immersed in wash water (Table 3). These tubers had been washed after digging and were washed again in distilled water at the beginning of the experiment. Nevertheless, when they were immersed in this second wash water for 5 min. they showed numerous lenticel infections 48 hr. after removal from the water. These results show that commercially washed potatoes may carry on their surfaces sufficient numbers of virulent soft-rot bacteria to later bring about lenticel infections if favorable environmental conditions are provided.

It has been shown in these studies that lenticel infections are readily obtained by placing uninjured tubers, inoculated by immersion in a water suspension of certain of the lenticel isolates, in moist chambers in which a high relative humidity is maintained. Since, however, no accurate data were available as to the effect of varying relative humidities and temperatures on the incidence of lenticel infection, it seemed desirable to make such determinations.

For this purpose, solutions of sulphuric acid and distilled water were prepared according to the table given by Stevens (21) for providing relative humidities of 70.4, 80.5, 90.0, 94.8, 98.2, and 100 per cent. Two lots each of White Rose and Bliss Triumph potatoes that had been previously stored at 61° and 72° F., respectively, for 24 hr. were used. Immediately upon removal from storage they were washed in tap water, dipped in 80 per cent alcohol, and dried in the air. Six tubers of each of these varieties were inoculated by immersing them for 5 min. in a water suspension of bacteria from an 18-hr.-old agar slant culture of an isolate from series 15, after which they were placed in moist chambers in which the various relative humidities were maintained. Unless otherwise indicated, controls consisted of an equal number of both varieties that had been immersed in distilled water for 5 min. before placing them in moist chambers. All of the inoculated and non-inoculated tubers were then placed in an environment where constant temperatures of 61° and 72° F. were maintained. The results of the experiment appear in table 4.

Lenticel infection did not occur within 72 hours at 61° and 72° F. at relative humidities of 70.4, 80.5, and 90.0 per cent. At a relative humidity of 94.8 per cent no infection occurred when the temperature was 61° F., but infection was visible after 48 hr. incubation when the temperature was 72° F. At a relative humidity of 98.2 per cent and a temperature of 61° F. both varieties showed some infection after 48 hr. At 100 per cent relative humidity and a temperature of 72° F. all of the Bliss Triumph and 50 per cent of the White Rose tubers were infected after 24 hr. incubation. None of the

control tubers at the various relative humidities and temperatures showed lenticel infection at the end of 72 hr. incubation. Infection of tubers did not develop so rapidly at 61° F. as when they were incubated at 72° F., even though relative humidities of 98.2 and 100 per cent were maintained.

The effect of still higher temperatures on the progress of infection was observed in some preliminary experiments when inoculated tubers were incubated at 80° F. and at a relative humidity of approximately 100 per cent. Under these conditions lenticel infection was often visible after 18 hr. incu-

TABLE 4.—*Influence of relative humidity and temperature on lenticel infection of potatoes by a lenticel isolate (from series 15)*

Variety	No. tubers inoculated[a]	Temperature (°F.)	Relative humidity (%)	No. tubers infected[b] after		
				24 hr.	48 hr.	72 hr.
Bliss Triumph	4	61	70.4	0	0	0
	4	72	Do	0	0	0
White Rose	4	61	Do	0	0	0
	4	72	Do	0	0	0
Bliss Triumph	4	61	80.5	0	0	0
	4	72	Do	0	0	0
White Rose	4	61	Do	0	0	0
	4	72	Do	0	0	0
Bliss Triumph	6	61	90.0	0	0	0
	6	72	Do	0	0	0
White Rose	6	61	Do	0	0	0
	6	72	Do	0	0	0
Bliss Triumph	6	61	94.8	0	0	0
	6	72	Do	0	3	4
White Rose	6	61	Do	0	0	0
	6	72	Do	0	1	2
Bliss Triumph	6	61	98.2	0	2	4
	6	72	Do	3	5	6
White Rose	6	61	Do	0	1	3
	6	72	Do	1	3	5
Bliss Triumph	6	61	100.0	0	3	5
	6	72	Do	6	6	6
White Rose	6	61	Do	0	2	3
	6	72	Do	3	4	5

[a] Tubers were inoculated by immersing them in a water suspension of bacteria for 5 min. In each lot an equivalent number of control tubers were immersed in distilled water for 5 min.

[b] None of the 128 control tubers of the two varieties had lenticel infections during 72 hr. incubation.

bation and after 24 hr. a high percentage of the lenticels on the tubers showed infection.

From these data on inoculation tests, under controlled temperature and humidity conditions, it would appear that few or no infections through lenticels will occur in the field or in transit within 3 days at a temperature below 72° F. and a relative humidity less than 94.8 per cent. Under moist or wet soil conditions, however, the above temperature and humidity conditions are frequently exceeded in some regions growing early potatoes, so that

time lag is not of practical importance in the occurrence of infection. Tubers not infected at loading time are not likely to become infected and develop decay during transit unless both the temperature and humidity are high. In commercial as well as in test shipments of nonrefrigerated cars it has been observed that most serious bacterial soft rot occurs at the quarter-length position which is the warmest and probably the most humid part of the load (14).

HISTOLOGICAL STUDIES

Histological studies were made of normal lenticels and those which were naturally or artificially inoculated. For this purpose, small portions of the desired tissue were removed from the tubers and were killed and fixed in a mixture of formalin-acetic acid-alcohol, embedded, and sectioned in the usual manner. Microtome sections 12 microns thick were stained with basic fuchsin with orange G as a counterstain.

Sections were prepared from naturally infected lenticels on tubers that had remained in an atmosphere of low relative humidity for seven days. As a result, the diseased area had lost its turgidity and the tissue surrounding the lenticels had become slightly sunken. A cross section through such a lenticel reveals several layers of periderm cells that have become separated from the parenchyma, resulting in the formation of a cavity or pocket, with suberized parenchyma cells below. Bacteria are found between the cells in this area (Fig. 3, A). A cross section through a naturally infected lenticel having a more advanced stage of decay is shown in figure 3, B. The periderm layer is sunken while the parenchyma cells beneath have become separated from it. Bacterial invasion has become extensive in the parenchyma. Suberized cells may be observed in the invaded area, while immediately beneath it new periderm has been laid down. Artschwager (1) has shown, in studies with potato, that suberization is prerequisite to wound-periderm formation. In the present studies of naturally infected lenticels, suberization invariably preceded wound-periderm formation.

In the preliminary studies of naturally infected lenticels difficulty was experienced in obtaining infected material that would reveal, upon cutting, early stages of bacterial infection. Studies were therefore made of material from artificially inoculated tubers. Sound potatoes were placed in a water suspension of one of the lenticel isolates for 5 hr., after which they were removed and placed in moist chambers. Infections, as indicated by swollen areas around lenticels, were apparent after 24 hr. (Fig. 2, A). Small portions of tissue containing the infected lenticels were removed from the tubers and were killed, fixed, and embedded in paraffin. Infected tissue was also removed from tubers 48 and 60 hr. after inoculation and fixed for further study.

A cross section of a normal lenticel (Fig. 4, A) of the variety Bliss Triumph shows a periderm covered by a thin, rough crust. The periderm is made up of 5 to 8 rows of cells arranged radially. The parenchyma consists of masses of loose, roundish cells.

FIG. 3. A. Cross section through naturally infected lenticel. Periderm has become separated from adjoining parenchyma. Suberized cells may be seen beneath. ×125. B. Cross section through naturally infected lenticel in a more advanced stage of decay. Bacterial invasion has become extensive in the parenchyma. Suberized cells may be seen in the invaded area. Immediately beneath new periderm has been laid down. ×125.
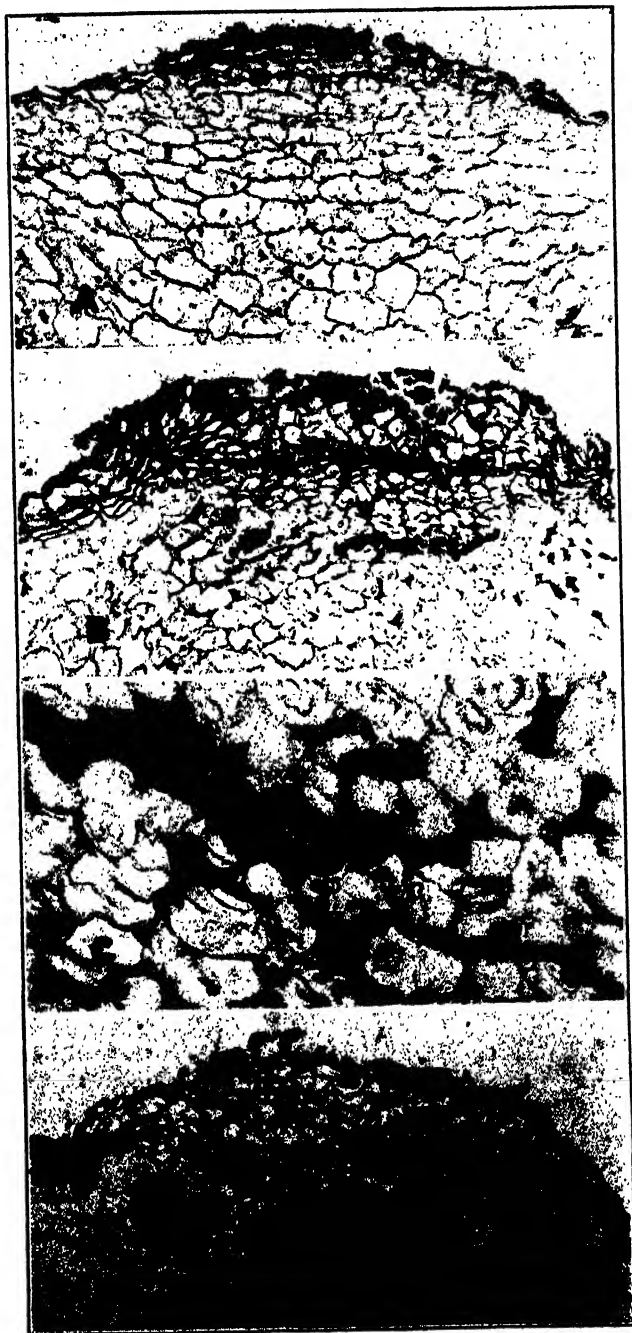
FIG. 4.   A. Cross section through normal lenticel.   × 135.   B. Cross section through infected lenticel 24 hr. after inoculation.   × 130.   C. Detail of 4, B, more highly magnified to show extent of bacterial invasion between parenchyma cells.   × 220.   D. Section through infected lenticel 48 hr. after inoculation.   Intercellular invasion resulting in some cell collapse has occurred.   × 135.

A cross section through an infected lenticel 24 hr. after inoculation is shown in figure 4, B, C. At this stage the bacteria have penetrated to a considerable depth into the lenticel and may be seen between the parenchyma cells. A section through an infected lenticel 48 hr. after artificial inoculation reveals bacterial invasion between cells. Numerous cells have become so disintegrated that masses of bacteria have coalesced along most of the width of the lenticel (Fig. 4, D). Tubers held under high humidity and moderately high temperature, when examined 60 hr. after inoculation, were generally in such a state of disintegration that suitable paraffin sections were not obtained. Free-hand sections, however, revealed that decay had advanced into the tuber often to a depth of 10 to 15 mm., resulting in almost complete destruction of parenchyma cells in the affected area. Under those conditions favoring rapid development of decay no evidence of protective new periderm formation was observed. This indicates that in commercial shipments received on the market with extensive soft rot following lenticel infection, conditions similar to those above may exist.

Tubers were artificially inoculated by immersion in water suspensions of bacteria and after 72 hr. incubation in moist chambers·were removed and placed in the open in the laboratory for 72 hr. Sections through infected lenticels on these tubers show essentially the same condition noted with naturally infected lenticels, *viz.*, a slight sunken area in the vicinity of the lenticel, with the separation of the parenchyma from the original periderm layer in the decayed area. Macroscopically, the decayed portion appears to be rather sharply delimited from the normal tissue (Fig. 2, E). Free-hand sections of tissues in this area, stained with ammoniacal gentian violet, reveal a definite suberized layer between the diseased and healthy tissues and the formation of new periderm beneath.

MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF THE ISOLATES

In these studies the organisms were isolated in pure culture by the dilution plate method. Cultures were maintained on potato-dextrose agar (pH 6.8) and in nutrient broth (pH 6.8). Single-colony isolations from cultures purified by the dilution-plate method were used in these as well as in the pathogenicity studies. All tests were run in duplicate.

Authentic cultures of *Erwinia carotovora* used for comparison in the studies included one from J. G. Leach designated W.Va. 2 in this paper; one from G. W. Keitt (Wis. 1); one from D. H. Rose (U.S.D.A. 3545); and three that the writers had isolated and proved pathogenic. Of the latter, two (3319 and 3470) were originally isolated from potatoes. One, designated S 27, was isolated from decayed spinach.

In the morphological, biochemical, and physiological studies of the various isolates, certain of the recommendations of the Committee on Bacteriological Technique, Society of American Bacteriologists, as given in the Manual of Methods of Pure Culture Study of Bacteria (20), were followed. The results of these studies are summarized in table 5.

Morphological characteristics. For determination of form and size, negative preparations from smears were made with 1 per cent nigrosine. Fisher and Conn's flagella stain (7) was used to determine the number and

TABLE 5.—*Morphological and physiological characteristics of bacteria used in pathogenicity studies*

Isolation series no.	No. isolates used	Chains[b]	Nitrates Reduction	Nitrates Gas	Gelatin liquefaction	Hydrogen sulphide	Diastatic action	Indole	Milk Curd	Milk Peptonization	Milk Gas	Dextrose Acid	Dextrose Alkaline	Dextrose Gas	Sucrose Acid	Sucrose Alkaline	Sucrose Gas	Lactose Acid	Lactose Alkaline	Lactose Gas
1	2	+	+	-	-	-	-	-	+	-	-	+	-	+	-	-	+	-	+	-
2	3	+	+	-	-	-	+	+	-	-	+	-	+	-	-	+	-	+	-
3	3	+	+	-	-	-	+	+	-	-	+	-	+	-	-	+	-
4	2		+	-	-	-	-	+	-	-	+	+	-	-	+	-
5	3		+	-	-	-	-	+	-	-	+	+	-	-	+	-
6	2	+	+	-	-	-	+	+	-	-	+	+	+	-	+	-	-	+	-
7	2	+	+	-	-	-	-	+	-	-	+	+	-	-	+	-
8	2	+	+	-	-	+	-	-	+	-	-	+	+	-	-	+	-
9	3	+	+	-	-	-	-	+	-	-	+		-	-	+	-
10	4		+	-	-	-	-	+	-	-	+	+	-	-	+	-
11	2		+	-	-	-	-	+	-	-	+	+	-	-	+	-
12	1		+	-	-	-	-	+	-	-	+	+	-	-	+	-
13	2	+	+	-	-	-	-	+	-	-	+	+	+	+	-	+	-
14	2		+	-	-	-	-	+	-	-	+	+	-	-	-	-
15	2	+	+	-	-	-	+	+	-	-	+	+	-	-	+	-
16	1		+	-	-	-	-	+	-	-	+	+	-	-	+	-
17	1	+	+	-	-	-	-	+	-	-	+	+	-	-	+	-
18	4		+	-	-	+	-	-	+	-	-	+	+	-	-	+	-
19	2		+	-	-	-	-	+	-	-	+	+	-	-	-	-
20[a]	2		-	-	+	+	+	+	-	-	+	+	-	-	+	-
21	2		+	-	-	-	-	+	-	-	+	+	+	+	-	+	-
22	3		+	-	-	-	-	+	-	-	+	+	-	-	+	-
23	2	+	+	-	●	-	-	+	-	-	+	+	-	-	+	-
24	4	+	+	-	-	+	-	-	+	-	-	+	+	+	+	-	+	-
25	2		+	-	-	-	-	+	-	-	+	+	-	-	-	-
26	2		+	-	-	-	-	+	-	-	+	+	-	-	-	-
27	1		+	-	-	-	-	+	-	-	+	+	-	-	-	-
28	2	+	+	-	-	-	-	+	-	-	+	+	-	-	+	-
29	2		+	-	-	-	-	+	-	-	+	+	-	-	+	-
30	1		+	-	-	-	-	+	-	-	+	+	+	+	+	+	-
W. Va. 25	1		+	-	-	-	-	+	-	-	+	+	-	+	-
Wis. 1	1		+	-	-	-	-	+	-	-	+	+	+	+	+	+	-
3545	1		+	-	-	-	-	+	-	-	+	+	+	+	+	+	-
3319	1		+	-	-	-	-	+	-	-	+	+	+	+	-
3470	1		+	-	-	-	-	+	-	-	+	+	+	+	+	+	-
S-27	1		+	-	-	-	+	+	-	-	+	+	-	+	-

[a] *Bacterium coadunatum.*
[b] In other morphological characters all isolates were motile, all were Gram-negative, and all were without capsules.

position of flagella. Gram reaction was determined according to Hucker and Conn's (8) modification of Gram's stain. Measurements of bacteria from the different series showed a variation in length from 1.5 to 4.0 μ and in width from 0.5 to 0.8 μ. Neither spores nor capsules were formed. Cells were mostly single or in pairs though an occasional chain was observed.

Biochemical characteristics. In the miscellaneous biochemical reactions the isolates were facultative anaerobes. In the tests on nitrate reduction, 7- and 10-day-old cultures were used. The alpha-naphthylamine-sulfanilic acid test was employed. The organism *Escherichia coli* Migula (Castellani and Chalmers) was used as a control. All of the isolates (except *Bacterium coadunatum*) reduced nitrates to nitrites without the production of gas. Liquefaction of gelatin was determined by means of stab cultures on plain gelatin. The cultures all caused a rapid liquefaction of this medium. In the studies of hydrogen sulphide production, strips of sterilized lead-acetate paper were hung over beef-extract broth cultures of the various isolates. Isolates in series 8, 18, 20, and 24 produced a faint blackening of the paper indicating the presence of hydrogen sulphide. None of the remaining isolates gave a positive reaction. *Escherichia coli*, which gave a positive test for hydrogen sulphide, was used as a control organism.

Tests for diastatic action were made according to the Eckford method (6). None of the isolates (except *Bacterium coadunatum*) were able to hydrolyze starch. The isolates were grown in Bacto-tryptophane broth and were tested for indole production. Cultures in series 2, 3, 6, 15, and 20 gave weakly positive tests. None of the other cultures gave positive reactions for indole. When *Escherichia coli* was used as a control organism, a positive test was obtained. In tests with litmus milk all the isolates produced an acid reaction and coagulated the milk. No peptonization occurred.

Carbohydrate fermentation. In the carbohydrate fermentation studies the peptone-free medium described by Ayers, Rupp, and Johnson (2), but with slight modifications as given by the Society of American Bacteriologists, was used (20). The fermenting action of the bacteria was tested in Smith fermentation tubes to which 1 per cent of the respective sugars had been added. Bromcresol purple at a concentration of 0.02 per cent was added as an indicator. In all of the fermentation studies, color change from purple to yellow was considered the indication of fermentation. The final reaction of the various sugars was also determined by the glass-electrode pH meter.

All of the isolates from the various sources formed acid in dextrose, sucrose, and lactose media. It was observed, however, during the course of the experiment that a wide variation in the rate of acid production existed among many of the cultures. As will be noted, *Bacterium coadunatum* formed acid in dextrose and lactose but not in sucrose.

The results of the morphological studies indicate that there are no essential differences in the morphology of the various soft-rot isolates. The studies show that, with the exception of minor variations in the biochemical and physiological reactions of certain of the isolates, the organisms are closely related. It is apparent also that they are closely related to the authentic cultures of *Erwinia carotovora* used. Since the pathogenicity studies have shown that the various soft-rot isolates have produced essentially the same type of decay, it is concluded that they are strains of *Erwinia carotovora*.

It is possible that environmental factors provided by packaging for shipment may favor the production of pycnidia. Such factors might include exclusion of light, the accumulation of moisture, and the products of respiration of the corms. But, as some of these shipments arrived in well-ventilated packages, it seems advisable not to exclude the probability that fruiting pycnidia are more common on the corms of gladiolus than has been thought.—DONALD P. LIMBER, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture, Hoboken, New Jersey.

Spotted Wilt of Broad Bean.—During the winter of 1937, attention was called to a serious disease of broad bean, *Vicia faba* L., which was accompanied by symptoms characteristic of the spotted wilt. In the field, a top necrosis of the infected plant was very pronounced. The top portion of the plant shrivelled, blackened, and dried while the lower portion remained green for some time.

The virus was collected from a diseased bean field and was transmitted readily to young tomato plants in the greenhouse. Extracts from young leaves of infected tomato were rubbed on broad-bean seedlings. Typical streak resulted and the percentage of infection was moderate. Young seedlings about 6 inches high were killed within two weeks after inoculation. On old plants, the early symptoms on the leaves were spots, about 1 mm. in diameter, which soon enlarged to form oval or oblong lesions about 1 to 1.5 cm. long with diffuse margin. Later the lesions developed dark-colored zones and blackened. Infected bean leaves first became pale green then grayish black. Yellowish discolored areas were occasionally seen along the necrotic lesions. The leaves curled slightly downward or inward along the tip or margin. Finally, the entire leaf shrivelled. Collapse of the upper leaves resulted in the top necrosis commonly found in the field. Eventually, the lower leaves became involved and the plant was killed by the virus.

On bean stems, the virus produced purplish red streaks of various length, sometimes the entire length of the stem. Streaks were found also on leaf petioles. These streaks gradually blackened and the stems shrivelled and dried. In the field, if plants were infected after pods had formed, the seed was usually small, shrivelled and grayish green with black necrotic spots on the seed coat. The seed seldom germinated to produce normal seedlings.

As a rule, progress of the disease was rapid. The leaves quickly lost their normal greenness, turning to dark gray and collapsing. The whole plant was then killed, or the tops died down except for small lateral shoots. Rarely did an infected plant resume growth after infection.

In the greenhouse the virus was readily transmitted to tomato, tobacco, pepper, and *Zinnia elegans* Jacq. by juice inoculation. It produced the characteristic bronzing of tomato leaves. Small or large necrotic lesions appeared on inoculated tobacco leaves, but the plant usually was not killed.

Brownish black necrotic lesions appeared on inoculated pepper plants and reddish circular lesions developed on inoculated cowpea leaves.

The virus was relatively short-lived in extract. It usually was inactivated after aging for 8 hours in extract at 22° C. The inactivation temperature was approximately 42° C. and the tolerance to dilution was up to 1 : 80,000. Taking all of these properties *in vitro* and the symptoms produced on the various hosts into consideration, the disease of broad bean herein reported is unquestionably caused by spotted wilt virus.

In Chengtu, Szechwan province, China, tomatoes are grown extensively by farmers and spotted wilt has been found in certain tomato fields. The localization of diseased plants in the bean fields suggests transmission of the virus into the field from neighboring tomato plants through the medium of some insect vector.

For many years, extensive surveys of broad-bean diseases have been made and it is the first time the writer has seen broad bean naturally infected with and severely attacked by spotted-wilt virus in the field.—T. F. Yu, Department of Plant Pathology, The University of Nanking, Chengtu, China.

A Rough-bark Disease of Pittosporum Tobira.—An apparently undescribed disease of *Pittosporum Tobira* Ait. has been under observation and study in central California at Berkeley and in southern California in West Los Angeles for several years. The disease has also been noted in Albany, near San Jose, in Riverside, and Montebello, and apparently is more prevalent in southern than central California.

The most damaging effect of the disease is necrosis of the outer bark which later loosens and sloughs off (Fig. 1, A). Not infrequently the necrosis extends so deep into the bark that the branch is girdled and killed. Plants so affected have only terminal clusters of a few, small, rolled leaves on the branches. The shrubs are smaller than normal and sometimes slowly die. In addition to the bark symptoms, this disease produces characteristic leaf symptoms (Fig. 1, B). Not infrequently necrotic spots are produced in leaves. Leaf symptoms are of several types. The mildest consists of chlorotic blotches with indefinite margins, yellow on young leaves, tan on old ones; sometimes green islands are included in the areas. Another occasional type has small, angular, yellow areas suggestive of Cercospora leaf spot.[1]

An oak-leaf or water mark pattern occurring particularly along the midrib is common. Ring spots with concentric yellow lines or moiré patterns are less frequent. Similar symptoms were seen on *Pittosporum viridiflorum* Sims at Montebello, and on variegated *P. Tobira* in West Los Angeles, but these plants were not included in this study. No virus-like symptoms have been seen by the writers on *P. undulatum* Vent.

The disease is carried in cuttings from affected plants but is not completely systemic since less than half of such cuttings ever produced symptoms

[1] Plakidas, A. G. Angular leaf spot of Pittosporum. Mycologia 32: 601–608. 1940.

though they were kept under observation for more than two years. It is
not carried in seed readily if at all. Fifty-three seedlings from seed of an
affected plant and twenty-two from a plant without symptoms were kept
under observation for a year or more without developing any symptoms.
Among some 200 seedlings on the Los Angeles Campus of the University, no
symptoms were seen during the first several years after planting. Although
the symptoms seemed obviously to be those of a virus disease, a small-scale
inoculation test was made by inarching an affected to a healthy plant of
Pittosporum Tobira. Both bark and leaf symptoms appeared on part of
the inoculated plant within 14 weeks and soon afterward throughout the
plant. Cions from a plant of *P. crassifolium* A. Cunn. having virus-like
symptoms in leaves but not in bark, and growing near affected *P. Tobira*,

FIG. 1.   A. Rough bark on an old plant of *Pittosporum Tobira*.   B. Leaf symptoms.

were grafted on healthy *P. Tobira*. After something over five months the
leaves of the inoculated *P. Tobira* began to develop symptoms quite similar
to those occurring naturally on that species but no bark symptoms had
appeared up to seven months from the time of inoculation.

Some indication of natural spread of the disease of *Pittosporum Tobira*
was seen in two gardens in Berkeley. In paired plants several years old
growing about 20 feet apart, one in each case bore symptoms when first
observed. After several years the second plant of each pair slowly devel-
oped symptoms. Evidence of natural spread has also been seen at Los
Angeles. On the other hand some of the 15-year-old plants near San Jose
show symptoms while others in actual contact with them do not. A similar
situation prevails in one planting at Berkeley.

It is not yet clear whether this disease of *Pittosporum Tobira* is related

to that which seems to have been transmitted to *P. Tobira* in France in 1887[2] or to that described for *P. daphniphylloides*.[3]

It should be possible to easily control the disease by care in selection of cuttings, by rogueing out affected plants, and by growing plants from seed.— H. EARL THOMAS and KENNETH F. BAKER, Division of Plant Pathology, University of California, Berkeley and Los Angeles.

A Fungicide for Protecting Lily Bulbs from Infection by Colletotrichum lilii.—The use of an eradicant fungicide for controlling bulb-borne infection of Easter Lily (*Lilium longiflorum* Thumb. var. *eximium*) bulbs by the black scale fungus *Colletotrichum lilii* Plakidas has been reported.[1] In more recent work it was found that while the treatment with Puratized N5E destroyed the disease organism in diseased tissue, treated bulbs received little or no protection from reinfection when planted in infested soil.

Since *Colletotrichum lilii* appears to live in the soil for several years, a treatment was sought which would protect the bulbs from infection by soil-borne inoculum. In preliminary greenhouse tests in 1944, infected bulbs, after treatment with Puratized N5X (phenyl mercuri triethanol ammonium lactate), were dusted with Arasan (tetramethyl thiuram disulfide) before being planted in potted, naturally infected soil. When dug about seven months later, all of the nondusted bulbs bore black scale lesions, while only 10 per cent of the Arasan-dusted bulbs were infected (Fig. 1). The disease indices (See footnote b in table 1) were 71 and 4 for nondusted and Arasan-dusted bulbs, respectively.

The use of Arasan as a protectant against soil-borne infection was then studied in field trials in the 1945–46 season in three different tests as follows:

1. A comparison of the Puratized N5E treatment with and without Arasan dust and Arasan dust alone with nontreated diseased bulbs in soil which had produced a severely diseased crop the previous year (1945).

2. Same as 1, except the soil had not been planted to Easter Lilies in 1945, but had produced a severely diseased crop in 1944. Nontreated bulbs were not included in this test, nor was Arasan used alone.

3. Clean disease-free bulbs dusted with Arasan were compared with untreated clean bulbs in soil which had produced severely diseased bulbs in 1945.

In experiments one and two, the Puratized N5E was used at a strength of 1–2000. The bulbs were treated for 48 hours in this solution. The Arasan was applied 24 hours after the bulbs were removed from the Puratized solution, and the bulbs were planted immediately. Where the Arasan was used without the Puratized treatment, the dry bulbs were dusted with Arasan immediately before planting.

[2] Carriere, E. A. Influence du greffon sur le sujet. Revue Horticole 59: 58–59. 1887.

[3] Milbrath, D. G. Probable virus disease of *Pittosporum daphniphylloides*. Monthly Bull. Calif. Dept. Agr. 29: 158–159. 1940.

[1] LeBeau, F. J. The eradicant action of a fungicide on Colletotrichum lilii in lily bulbs. Phytopath. 36: 391–393. 1946.

Experiments 1 and 2 were run in Plaquemines Parish and experiment 3 was located in Terrebonne Parish.

The results obtained are summarized in table 1.

Photographs of representative bulbs from each of the first three treatments of experiment 1 are shown in figure 1. It is evident that the Pura-
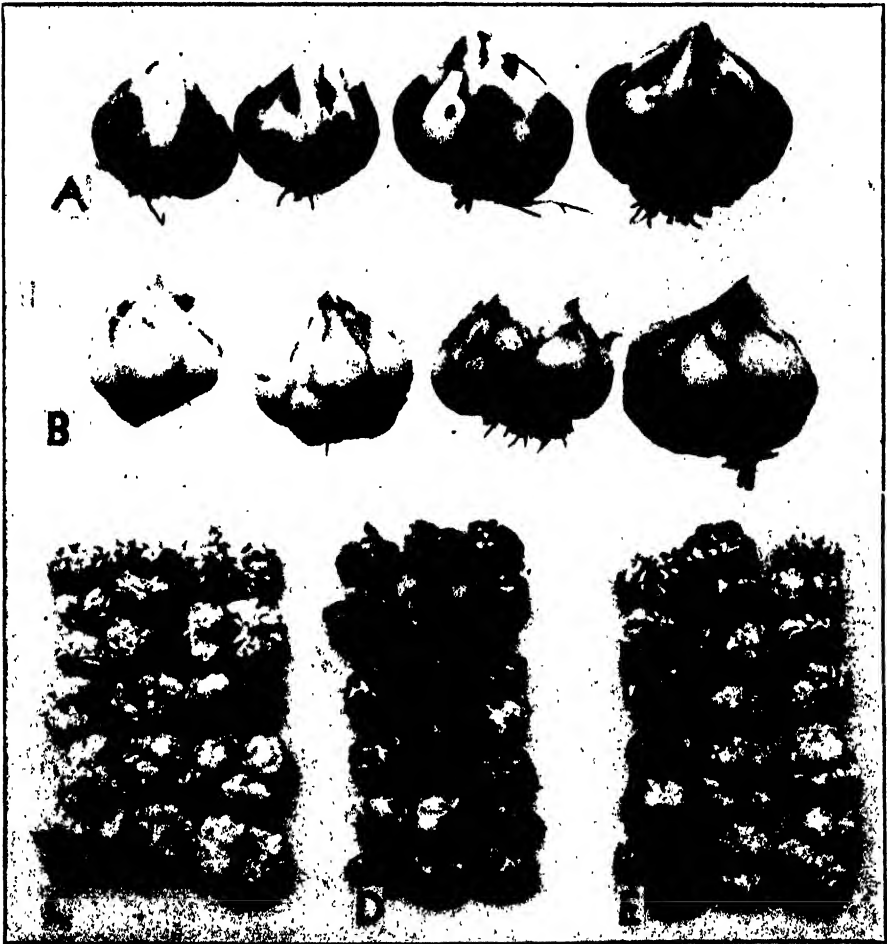
FIG. 1.   Effects of Arasan and Puratized N5E on the development of black scale on lily bulbs planted in infested soil.   (A) Infected bulbs treated with Puratized N5E only and grown in infested potted soil for 7½ months.   (B) Same as A except Arasan was dusted on the bulbs after the Puratized N5E treatment.   (C) Diseased bulbs receiving both treatments before being planted in infested soil in the field, and harvested 11 months later.   (D) Same as C but with no treatment before planting.   (E) Same as C except that only the Puratized N5E treatment was used.

tized N5E alone did not protect the bulbs from reinfection, while Arasan alone was not effective since it did not possess sufficient eradicant action to destroy infection already in the bulbs. The combined treatment, however, was highly effective. When clean bulbs were used, the Arasan treatment

TABLE 1.—*The effects of different treatments on the development of black scale on Easter Lily bulbs grown in infected soil*

Experiment No.	Treatment	No. of bulbs[a]	No. of bulbs in disease classes				Disease index[b]
			Clean	Mild	Mod.	Severe	
1	None	100	0	0	7	93	97.7
1	Puratized N5E	232	76	111	49	68	48.9
1	Puratized N5E followed by Arasan	250	222	24	4	0	4.3
1	Arasan	100	0	24	35	41	72.3
2	Puratized N5E	900	545	135	124	106	26.0
2	Puratized N5E followed by Arasan	672	610	32	20	10	5.1
3	None	134	46	40	20	28	41.0
3	Arasan	150	131	16	3	0	4.9

[a] Results are from 3–4 replications with the exception of the nontreated lot and the Arasan treatment in experiment 1. Infected bulbs were used for experiments 1 and 2; disease-free bulbs were used for experiment 3.

[b] Clean, mild, moderately and severely diseased bulbs were given numerical values of 0, 33.3, 66.6, 100, respectively. The disease index for any given treatment was obtained by summing the products of the numbers of bulbs in each class and the numerical values of the classes and dividing by the total number of bulbs in the treatment.

alone sufficed to prevent the development of black scale in infected soil.— F. J. LeBeau, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana.

The Effects of Carborundum in Inoculating Bean Plants with Bacteria.— In experiments reported elsewhere,[1] the carborundum technique for inoculation with viruses[2] yielded excellent results in inoculations with the wound parasite *Pseudomonas ribicola* Bohn and Maloit on *Ribes aureum* Pursh. To study the effect of carborundum in inoculations with bacteria that attack host leaves through stomata, the susceptible Black Valentine variety of kidney bean, *Phaseolus vulgaris* L., was inoculated with *Pseudomonas phaseolicola* Dowson and *Xanthomonas phaseoli* (E. F. Sm.) Dowson. Pertinent literature on the latter diseases was reviewed by Harter and Zaumeyer.[3]

Fully expanded primary leaves and first trifoliate leaflets were dusted with 300-mesh carborundum and rubbed with a cotton pad wet with water or with a suspension of one or the other of the parasitic bacteria. Paired primary leaves or first trifoliate leaflets on the same plants were similarly rubbed but without the application of carborundum. The inoculations were made on seedlings in individual pots in the greenhouse without the use of a moist chamber. An individual plant was used for only one type of inoculation.

The water checks did not develop any infection, but those dusted had

[1] Bohn, G. W., and J. C. Maloit. Inoculation experiments with *Pseudomonas ribicola*. Phytopath. **35**: 1008–1016. 1945.

[2] Rawlings, T. E., and C. M. Tompkins. Studies on the effect of carborundum as an abrasive in plant virus inoculations. Phytopath. **26**: 578–587. 1936.

[3] Harter, L. L., and W. J. Zaumeyer. A monographic study of bean diseases and methods for their control. U. S. Dept. Agr. Tech. Bul. 868. 1944.

small brown flecks that resulted from mechanical injury. In the inoculations with *Pseudomonas phaseolicola,* 1 of 30 dusted leaves or leaflets and 3 of 45 nondusted ones were free from infection; spots were considerably more numerous on the dusted organs (Fig. 1). In the *Xanthomonas phaseoli* series, 2 of 5 nondusted leaves escaped infection while all of the dusted leaves developed severe infection. Similar results were obtained with plants inoculated in the morning, in the afternoon, and at night.

The effects of adding carborundum to suspensions used to spray Black Valentine bean plants in the field at Cheyenne, Wyoming, were studied in 1943. In the control block alternating rows were sprayed with tap water

FIG. 1. Leaflets of the kidney bean, not dusted at left and dusted at center and right, rubbed with a cotton pad wet with a suspension of *Pseudomonas phaseolicola.*

or with a suspension of 300-mesh carborundum in tap water (20 g. in a liter). In the test block, odd-numbered rows were sprayed with a ½ dilution in tap water of beef-extract-dextrose-broth cultures of *Pseudomonas phaseolicola;* even-numbered rows were sprayed with the same suspension with carborundum added. After spraying between 6 and 7 p.m. on July 22, a mist was maintained over the plants for 2 hours with an overhead-spray irrigation system.

After 20 days, numerous bacterial spots had developed on all plants sprayed with the bacterial suspensions. No apparent difference in numbers of spots resulted from the use of bacterial suspensions containing carborundum and those lacking it. No injury was seen on leaves of the controls sprayed with water either with or without the carborundum. Apparently,

carborundum applied in this manner caused few additional infection courts for the bacteria. It is obvious that carborundum is unnecessary to obtain abundant infections with stoma-invading parasites if experimental conditions favor abundant ingress through stomata.

These experiments suggest that carborundum powder may be useful in inoculations with stoma-invading parasites under conditions that do not favor ingress of the parasite through stomata but is of little value under conditions that do favor such ingress. These experiments, together with those on bacterial spot of currant cited above, suggest that carborundum may be found most useful with wound-parasites and with hosts that have hard, glabrous, thickly cutinized leaves.—G. W. BOHN, formerly Associate Plant Pathologist, Cheyenne Horticultural Field Station, and J. C. MALOIT, formerly Agent, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

Efficacy of Certain Soil Fumigants and Fertilizers against Crown Rot in Annual Larkspur Caused by Sclerotium rolfsii.[1]—A commercial flower grower in the Rio Grande Valley of Texas has experienced extensive losses from crown rot caused by *Sclerotium rolfsii* Sacc. in his annual larkspur (*Delphinium ajacis* L.) for several years. Treatments, in August, 1944, of parts of one field with several materials, including chlorpicrin and Iscobrome No. 2, showed promising control of *S. rolfsii*. In December of 1945 fertilizers and soil fumigants were applied in plots in the part of this field that was known to be heavily infested with sclerotia of *S. rolfsii*. Soil fumigants were included to determine whether the 1944 observations could be substantiated, and the fertilizers were included to determine whether control of *S. rolfsii* similar to that obtained by Leach and Davey in California[2] could be obtained in the Rio Grande Valley.

There were 12 treatments (Table 1) applied to plots 9 feet wide by 18 feet long, and each treatment was randomized in each of 6 blocks. The soil in this field was very low in organic matter, had a pH of 5.6 when tested in 1944 (but probably was more alkaline in 1946), and consisted of a very fine sand and clay. Water used for irrigation was alkaline. The soil fumigants were applied December 12 and 13, 1945, chlorpicrin with a Larvajector, and the other fumigants with a Mack Anti-Weed gun. The soil was very dry at the time of application (1.9–2.2 per cent moisture) and the soil temperature was 73–74° F. at the 6-inch depth. No water seal was applied. The Uramon and ammonium nitrate in treatments 10 and 11 were broadcast uniformly over the plots December 11. A period of cold weather, locally known as a "Norther," prevailed from December 10 to 13, during which time it was cold and cloudy with occasional drizzling rain and with air temperatures of 35–65°. This probably tended to retard volatilization of the gases from the

[1] Thanks are due J. O. Davis, Manager, Weatherford's Farms, Linn, Texas.
[2] Leach, L. D., and A. E. Davey. Reducing southern sclerotium rot of sugar beets with nitrogenous fertilizers. Jour. Agr. Res. [U.S.] 64: 1–18. 1942.

soil.   On January 9, 1946, seeds of larkspur, variety Imperial Los Angeles, were seeded in 3 rows running the length of each plot.   The fertilizers in treatments 5 to 9 were divided into 3 equal lots and applied as sidedressings on the following dates: January 30, February 20, and March 20, 1946.   Complete flower-production records could not be obtained from these plots, but the healthy larkspur plants in the middle row of each plot were counted April 5, 1946, when plants were just beginning to flower, and large numbers had been killed by or were dying because of *Sclerotium rolfsii*.   The data are in table 1.

TABLE 1.—*Number of healthy larkspur plants at flowering following certain soil-fumigant and nitrogen-fertilizer treatments for the control of* Sclerotium rolfsii *in the Rio Grande Valley of Texas*

Materials			Applications		Rate per acre		Healthy larkspur plants
Soil fumigants		Amt. per injection (cc.)	Spacing of injections (inches)	Fumigant			Number[c]
				(gal.)	(lb.)		
1.	Chlorpicrin	2.5	12	28.7	402		237
2.	Carbon disulphide	20.0	18	102.2	1073		185
3.	Iscobrome No. 2[a]	5.0	12	57.5	575		199
4.	ETN mixture[b]	10.0	18	51.1	470		170
Nitrogen fertilizers		Method and time		Fertilizer (lb.)	N (lb.)		Number[c]
5.	Ammonium sulphate	3 sidedressings at monthly intervals		735.0	150		144
6.	Ammonium nitrate	do		450.0	150		248
7.	Cyanamid	do		712.5	150		155
8.	Uramon	do		356.8	150		154
9.	do	do		712.5	300		189
10.	do	Broadcast before planting		950.0	400		18
11.	Ammonium nitrate	do		1200.0	400		19
12.	Control, not treated						115

[a] Xylol, 60 per cent; methyl bromide, 15 per cent; and chlorpicrin, 25 per cent.
[b] Ethylene dibromide, 15 per cent; tetrachlorethane, 20 per cent; naphtha thinner, 65 per cent.
[c] Least difference necessary for significance with odds of 19:1, 66.7; odds of 99:1, 88.5.

All of the materials resulted in an increased number of healthy plants, with the exception of Uramon (a urea compound) and ammonium nitrate broadcast at the rate of 400 pounds per acre before planting.   These two treatments were injurious to the growth of the plants.   Of the fumigants, chlorpicrin gave excellent control of *Sclerotium rolfsii* as evidenced by the number of healthy plants.   Iscobrome No. 2 and carbon disulphide gave fair control.   Three sidedressings with ammonium nitrate totaling 150 lb. nitrogen per acre also gave excellent results but, with the exception of Uramon at 300 pounds of nitrogen per acre, none of the other nitrogen-carrying fertilizers was more effective statistically than no treatment.

Leach and Davey reported equally effective control of *Sclerotium rolfsii* in sugar beets in California with equivalent amounts of nitrogen from ammonium sulphate, anhydrous ammonia, calcium nitrate, and Cyanamid. In laboratory tests, however, low concentrations of ammonia in aqueous solution were toxic to mycelium and sclerotia of *S. rolfsii*, ammonium sulphate in alkaline solutions were mildly toxic to mycelium, and calcium nitrate was nontoxic. They were unable to explain this inconsistency satisfactorily. In the Rio Grande Valley tests ammonium sulphate did not control the disease whereas equivalent amounts of nitrogen from ammonium nitrate resulted in good control. Possibly the nitrate ion is more effective than the ammonium ion in the Rio Grande Valley soil, or the ammonium nitrate may have remained available over a longer period of time, resulting in greater inhibition of growth of the *S. rolfsii*. There is also the possibility that ammonium nitrate causes a greater change in the soil microflora, antagonistic to *S. rolfsii*, in Texas than do the other fertilizers.—W. D. McCLELLAN, Division of Fruit and Vegetable Crops and Diseases, Agricultural Research Administration, United States Department of Agriculture, Beltsville, Maryland.

THE ISOLATION AND BEHAVIOR OF BACTERIA–FREE CROWN–GALL TISSUE FROM PRIMARY GALLS OF HELIANTHUS ANNUUS

R . S . D E R O P P [1]

That bacteria are frequently absent from secondary crown-gall tumors of sunflower was demonstrated clearly by Braun and White (2). The same phenomenon was observed earlier by Smith, Brown, and McCulloch (7), who recorded several instances in which they were unable to isolate *Phytomonas tumefaciens* from secondary galls on Paris daisy. These workers, however, attributed their failure to faulty technique or to the cultural peculiarities of the parasite. Significant observations were also made by Jensen (3), who studied the naturally occurring galls on beets. Such galls were almost un-questionably caused by *Phytomonas tumefaciens* and showed, when grafted into healthy plants, the same capacity to produce tumors as was later demonstrated by White and Braun (9), using bacteria-free crown-gall tissues from secondary galls of sunflower. Jensen concluded "The tumours in beets are undoubtedly caused by *Bacterium tumefaciens*, but in older tumours, the bacteria die off, and careful investigation of a large number of spontaneous tumours from mangels and sugar beets, taken up in autumn, led only in a single instance to isolation of the bacterium." The work to be described in this paper was linked with these findings of Jensen. It was designed to ascertain whether primary galls on sunflower, tobacco, and tomato ever be-came free of bacteria, thus forming a source of bacteria-free tumor tissue such as was isolated from secondary galls on sunflower (9) or from galls on heat-treated periwinkle plants (1, 8).

EXPERIMENTAL WORK

Plants of sunflower (*Helianthus annuus* L.), tomato (*Lycopersicon esculentum* Mill.), and tobacco (*Nicotiana tabacum* L.) were inoculated at 4 to 6 weeks of age with a 48-hour broth culture of *Phytomonas tumefaciens*, strain A6. The inoculation was made by means of a spear-headed needle dipped in the culture and inserted into one of the lower internodes of the plant. After 6 weeks' further growth, well-developed galls were produced at the site of inoculation. Internodes bearing these galls were rendered sterile by careful removal of the epidermis. The outer part of the gall was cut away by means of a sterile scalpel. The internode in the region of the gall was then cut into a series of sections about 3 mm. thick which were transferred to slopes of sucrose agar medium having the same composition as that employed by White and Braun (9) for the cultivation of secondary tumors.

[1] Research Officer, Agricultural Research Council, Great Britain, and Visiting In-vestigator, Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, New Jersey.

The behavior of these isolated fragments of tissue was closely observed during four weeks. From the fragments of tomato and tobacco tissue outgrowths of bacteria were observed after the cultures had been incubated from 3 to 4 days. The bacteria formed thick, mucilaginous films which covered the surface of the agar and proved, on inoculation into sunflower,
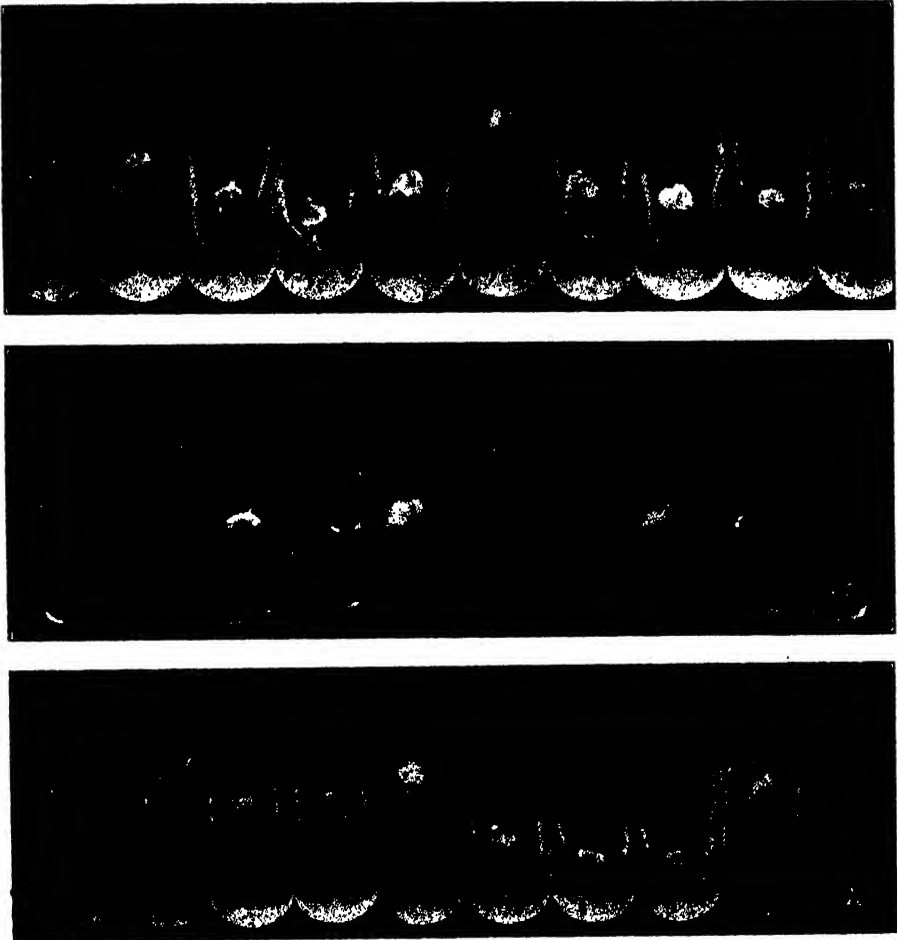
FIG. 1. *a*. Serial segments through a gall-bearing internode of sunflower after 6 days' culture *in vitro*. White masses of tumor tissue are visible in the fifth through the eighth segments. Bacteria are absent throughout. *b*. Same series of segments as in *a*, after 26 days' culture *in vitro*. Tumor tissue is more clearly visible, accompanied by the development of roots from normal tissue. *c*. Serial segments through a gall-bearing internode of sunflower after 6 days' culture *in vitro*. Bacteria were present in segments from above and below the gall and in one segment from the center of the gall, though absent from other segments. (× 0.45.) (Photographs by J. A. Carlile.)

to be *Phytomonas tumefaciens* in every instance. In both tomato and tobacco it appeared that the parasite had spread throughout the gall and extended for some distance into healthy tissue above and below it. None of

the stem cultures prepared from these plants proved to be free of the parasite.

When stem segments from infected sunflowers were cultured on nutrient agar in the same way a different result was obtained (Fig. 1, *a*). In 11 plants out of a group of 20 no bacteria could be isolated from any one of the series of stem segments. Even segments which had been excised from the center of the gall showed no signs of the organism. Segments from this region, however, frequently developed outgrowths of a white, friable tissue which grew rapidly, soon attaining a size greater than that of the original fragment. This white, disorganized tissue closely resembled, in appearance and mode of growth, the tumor tissue isolated by White and Braun (9) from secondary galls on sunflower. The masses of tumor tissue can be seen growing out of the stem fragments in figure 1, *b*. From the normal tissue of such fragments a copious outgrowth of roots generally took place. Sections of these segments indicated that the roots arose in normal tissue probably under the stimulus of a root-forming substance generated by the tumor tissue (See Fig. 2, *b*).

In 9 out of the 20 sunflower plants examined bacteria were isolated from some or all of the stem segments. The distribution of bacteria amongst these segments was often irregular (See Fig. 1, *c*), the organisms being frequently present in the stem above and below the gall though absent from the gall region itself. It must be emphasized, however, that the entire gall was not cultured in these cases, since, in order to obtain sterile tissue, the external surface of the gall had to be removed. It has been stated by Robinson and Walkden (4) that *Phytomonas tumefaciens* multiplies on the external surface of galls; and Braun and White (2), who tested one hundred primary galls from sunflowers, recovered bacteria from all of them. These results, therefore, only indicate that the interior of the gall on sunflower is often free of bacteria though it may and usually does contain altered tissue capable of autonomous growth.

Those segments of sunflower stem which contained tumor tissue but were free from bacteria were transferred to fresh agar in 250-ml. flasks after a period of one month. The tumor tissue continued to proliferate rapidly, giving rise to large tissue masses containing both normal and tumor tissue held in the agar by a considerable growth of roots (Fig. 2, *a*). After a further month of culture these masses were removed from the flasks and the tumor tissue was separated from the normal tissue as far as possible; fragments of it were then transferred to agar slopes. From fragments of three different sunflower plants three new strains of bacteria-free tumor tissue were isolated. These were designated P_I, P_{II}, and P_{III}. The behavior of these fragments in culture was fundamentally different. P_I and P_{III} both grew freely on the surface of the agar as whitish, friable masses tending to become brown with age. Strain P_I at first tended to produce roots (Fig. 2, *c*), though this tendency later disappeared and may only have been due to contamination of the tumor tissue with normal tissue. Strain P_{II}, however,

FIG. 2. *a.* Mixed normal and tumor tissue with roots cultured from a bacteria-free segment of primary gall on sunflower. *b.* Longitudinal section of the segment from gall-bearing internode of sunflower showing production of root initial in normal tissue probably resulting from stimulus by central mass of tumor tissue. *c.* Root production in an early subculture of strain P_i derived from a primary sunflower tumor. (×1.2; ×14.8; ×9.3.) (Photographs by J. A. Carlile.)

tended to grow down into the medium and the consistency of the tissue produced by the strain was firm and woody. The difference in structure of the soft as compared with the woody strain can be seen in figure 3, *a* and *b*. All these strains of tissue, when grafted into intact sunflower plants by the method described by White and Braun (9), proved capable of autonomous growth, giving rise to large tumors on the stems of the host plants.

SUMMARY AND CONCLUSIONS

Tissue isolated from crown galls on tobacco and tomato was always found to contain viable organisms of *Phytomonas tumefaciens* in large numbers. The interior tissues of primary galls on sunflower were frequently

FIG. 3. *a.* Structure of the woody type of tumor tissue from strain P_u isolated from a primary tumor. *b.* Structure of the soft translucent tumor tissue from strain P_1 isolated from a primary tumor. (× 11.6.) (Photographs by J. A. Carlile.)

found to be free of bacteria and new strains of bacteria-free crown-gall tissue could be obtained from this source. Tumor tissue obtained in this way was generally mixed with normal tissue, and tended to throw out roots for some time after its original isolation. With more prolonged culture this tendency disappeared. The roots probably arose from fragments of normal tissue embedded in the tumor tissue and stimulated into activity by growth substances generated by the tumor tissue. It does not seem likely that roots could be generated out of tumor tissue itself as it has already been shown (5) that pure tumor tissue does not respond to root-forming hormones such as indole acetic acid. This inability of tumor tissue to differentiate into organs was also commented on by Smith (6), "The tumor cell is a disoriented degenerate

cell . . . and I know of no evidence going to show that it can develop subsequently into normal tissues, organs, or the whole plant; on the contrary, it tends steadily towards decay."

Two types of tumor tissue were isolated from the primary galls, a hard, woody type with a fairly well-defined internal structure, and a soft, translucent type. On prolonged culture the woody type of tissue tended to change into the soft, translucent type.

AGRICULTURAL RESEARCH COUNCIL, GREAT BRITAIN,

AND

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,

PRINCETON, NEW JERSEY.

LITERATURE CITED

1. BRAUN, ARMIN C. Studies on tumor inception in the crown-gall disease. Amer. Jour. Bot. **30**: 674–677. 1943.
2. ————, and P. R. WHITE. Bacteriological sterility of tissues derived from secondary crown-gall tumors. Phytopath. **33**: 85–100. 1943.
3. JENSEN, C. O. Investigations upon certain tumour-like formations in plants. Den K. Veterinaer- og Landbohøjskole, Aarsskrift. **1918**: 91–143. 1918.
4. ROBINSON, WILFRID, and H. WALKDEN. A critical study of crown gall. Ann. Bot. **37**: 299–324. 1923.
5. DE ROPP, R. S. The response of normal plant tissues and of crown-gall tumor tissues to synthetic growth hormones. Amer. Jour. Bot. **34**: 53–62. 1947.
6. SMITH, ERWIN F. Effect of crowngall inoculations on Bryophyllum. Jour. Agr. Res. [U.S.] **21**: 593–598. 1921.
7. ————, NELLIE A. BROWN, and LUCIA McCULLOCH. The structure and development of crown gall: a plant cancer. U. S. Dept. Agr., Bur. Plant Indus. Bull. **255**. 1912.
8. WHITE, PHILIP R. Metastatic (graft) tumors of bacteria-free crown-galls on *Vinca rosea*. Amer. Jour. Bot. **32**: 237–241. 1945.
9. ————, and ARMIN C. BRAUN. A cancerous neoplasm of plants. Autonomous bacteria-free crown-gall tissue. Cancer Research **2**: 597–617. 1942.

ASCOCHYTA BLIGHT AND LEAF AND POD SPOT OF BROAD BEAN IN CHINA

T . F . Yu [1]

INTRODUCTION

In the spring of 1926, when the writer began a study of the diseases of broad bean, *Vicia faba* L., in Nanking, China, special attention was given to the blight and leaf and pod spot of beans caused by *Ascochyta pisi* Lib. The disease is less prevalent than the red-spot disease caused by *Botrytis fabae* Sard. and Cercospora leaf spot caused by *Cercospora fabae* Fautr., as it, in general, is restricted to certain fields and bean-growing districts. It is, however, very destructive, because it blights bean plants and withers the pods.

DISTRIBUTION AND ECONOMIC IMPORTANCE

The disease has been found by the writer in Kiangsu, Chekiang, Anhwei, Kiangsi, Hupeh, Szechwan, and Yunnan provinces. To judge from the wide range of conditions under which the disease has already been found, there appears to be no reason to doubt that it will be found in practically all the important bean-growing regions in China.

Outside of China, the disease has been reported in Italy (1), Spain (2), England (8), United States (12), Germany (9), East Africa (6), Cyprus (7), and Japan (3).

Heavy losses were caused by the fungus to broad beans in many sections of Kiangsu in 1934. Diseased plants yielded less than one-half the normal crop. From 1934 to 1937, the disease appeared in many bean-growing regions along the Yangtze river. The losses were generally slight except in certain places where young plants had been blighted. In 1940, an epiphytotic was observed in the higher bean lands near Chenkiang, Yunnan. Affected plants were stunted and there was a general yellowing of foliage. Loss ranged from 80 to 100 per cent of the crop.

Losses from the disease are due to destruction of plants, low yield of affected plants, and to poor germination of affected seed. When the disease appears early in the spring, young growing plants may be severely attacked. In this case, pods wither before they mature. Sometimes, the blighting of certain shoots of a plant prevents pod set. Spotting of seed renders them unfit for use as a green vegetable.

REVIEW OF LITERATURE

The blight, and leaf and pod spot of broad bean, has been attributed to either *Ascochyta pisi* Lib. or *A. fabae* Speg. by various investigators. *Ascochyta pisi* was first described in 1830 by Libert (4) as the cause of blight of

[1] Formerly Professor of Plant Pathology, The University of Nanking, Nanking, China.

Pisum sativum L.   In 1899, Spegazzini (11) described *Ascochyta fabae*, the cause of a dark yellow to brown leaf spot on *Vicia faba* L. from Argentina. This species of *Ascochyta* is characterized by large spores.

The *Ascochyta* species on leguminous plants were investigated by Sprague (12).   In cross-inoculations, *Ascochyta pisi* from pea was able to infect many species of *Vicia*, including broad bean, and the *Ascochyta pisi* from broad bean was able to infect 7 out of 10 species of *Vicia*, including *V. atropurpurea, V. cracca, V. dasycarpa, V. ervilia, V. monanthis, V. villosa,* and *V. sativa*.   The cultural characters were very similar for the *Ascochyta pisi* isolated from peas and from beans.   The spores of the fungus from broad bean were larger than those of the fungus from pea.   Relative to the spore size of *Ascochyta* on leguminous plants, Sprague has pointed out that there are transitional types between those on pea and those on broad bean.   He also showed that the difference of symptomatology produced by *A. pisi* from broad bean on other vetches from that produced by *A. pisi* from pea was due to host reaction.   As a result of this investigation, Sprague considered that most of the *Ascochyta* species on *Vicia* in North America are similar to or identical with *A. pisi* Lib. on peas and that the species on broad bean is a form of *A. pisi* resembling *A. fabae*.

Rathschlag (9) studied the *Ascochyta* parasitizing broad beans in Germany.   The symptoms produced by his fungus corresponded on the whole to the "dark type" produced by *Mycosphaerella pinodes* (Berk. and Blox) Stone while the light-colored center of the lesion more closely resembled the "light-type" produced by *A. pisi*.   In cross-inoculations, Rathschlag found that the fungi on pea and on broad bean were able to attack each other's hosts, but infection was uniformly more severe on the host from which the fungus was originally isolated.   Thus there was a certain degree of specialization, but by no means a strict one within the fungus.   Regarding spore size, he found that when *A. pisi* from pea was grown on broad bean, spores were slightly larger than they were on pea; when the fungus from broad bean was grown on pea, the spores were small.

Sattar (10) studied the fungi associated with blight of certain cultivated leguminous plants in India.   Although no *Ascochyta pisi* isolated from broad bean was included in his investigation, he supported Sprague's view that the form on broad bean is a variety of the *A. pisi* Lib. occurring on peas.

On the other hand, Ludwig (5) inoculated species of *Phaseolus* and *Vicia* with *Ascochyta pisi* from peas and obtained uniformly negative results except on peas.   On broad bean the fungus produced bluish spots with no trace of mycelium or spores.

SYMPTOMS OF THE DISEASE

The fungus attacks the leaves, stems, and pods of broad beans in the field. On the leaves, the spots are large, definite, circular to oblong, 2–22 × 2–16 mm. in diameter, with light-colored center and a dark or red border.   They may be zonate and irregular, with a tendency toward shot-hole.   The color

of the center of the spot is influenced to a certain extent by environment. In a dry season, as in Yunnan, the center is almost white. Under moist conditions, it may be gray or dirty white. In general, it is a light color. Spots occur at any point on the leaf surface. Sometimes the leaf tissue outside of the spot proper is blackened to form large dead areas of various size. Spots may also coalesce. In dry seasons, the affected leaves have a general yellowing, or plants are more or less defoliated.

Spots on leaves are at first dark brown, but soon develop the light center and dark red border. Pycnidia then develop and are either scattered over the spot or arranged in circles on the light center. They are reddish-brown

FIG. 1. Spots produced by *Ascochyta pisi* on leaves of broad bean in the field.

and very prominent. The various symptoms produced by the fungus on the leaves are shown in figures 1 and 3.

On stems, the lesions (Fig. 2) are circular, elongate, or oval, with gray centers and red margins. They are usually deeply sunken in the host tissue. Sometimes, large black areas without distinct demarcations occur. The lesions may attain a length up to 25 cm. In severe cases, stems are broken and die. Pycnidia are scattered over the diseased areas.

Spots on the pods (Fig. 2) are circular or oval and dark brown with black edges. They are usually deeply sunken in the host tissue. Lesions on the line of dehiscence of the pod are usually oval or a short spindle shape. Sometimes, lesions may assume such dimensions as to involve a large portion of the pod. When the attack of the fungus is severe, the pods shrivel. Consequently, only small immature seeds are produced. Diseased seeds may

be shrivelled or of greatly reduced size, dependent on the stage of development at which they are attacked by the fungus. Brown or black lesions are on the seed coat and severely infected seeds usually germinate poorly.

THE CAUSAL FUNGUS

The mycelium of the fungus within the host tissue varies greatly in
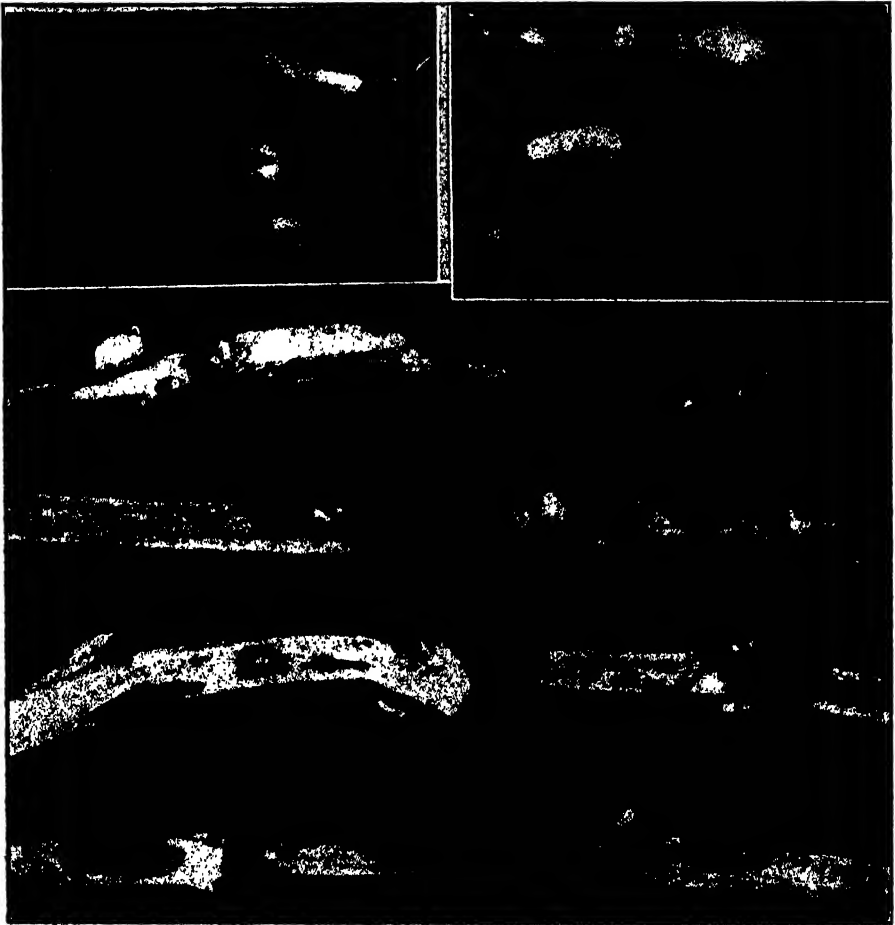
FIG. 2. Lesions produced by *Ascochyta pisi* on pods and on stems of broad bean in the field.

diameter. Hyphae are usually intracellular, but sometimes intercellular. On culture media they are hyaline and richly branched.

Pycnidia, which occur gregariously at the center or in circular zones on the spot, are prominent, globose-depressed, light brown, ostiolate, and $95-270 \times 111-301 \mu$ in diameter, with the average being $172 \times 178 \mu$. Spores are oblong, straight or sometimes curved, hyaline, 1- or rarely 2- or 3-septate, and measure $1.4-30.4 \times 3.8-7.9 \mu$, with an average of $17.9 \times 5.9 \mu$. The perfect stage of the fungus has not been observed.

Conidia germinate very rapidly in water at 20° C. More than 95 per cent of the spores germinate within 24 hr., by the production of germ tubes from one or both cells either simultaneously or in succession. Within 48 hr. as many as 4 germ tubes were observed coming from a single spore. Conidia germinate over a wide range of temperature, from 14° to 32° C. The optimum was about 22° C.

CULTURAL CHARACTERS

The growth of the fungus was moderate on both the tube and plate cultures. Pycnidia were produced on all of the media tested and were especially abundant on broad bean-seed agar and sterile broad-bean stems and pods. Cultures that originated from spores produced pycnidia more readily

FIG. 3. Leaf spots produced by *Ascochyta pisi* in dry seasons. Note the white centers.

than those originating from mycelium. With consecutive transfers of the fungus from mycelial cultures the pycnidia became more-sparse.

Growth in oatmeal-agar tubes was moderate, compact, and olivaceous green. On cornmeal agar, the growth was less compact. Scanty grayish white growth was found on broad-bean agar. On potato-dextrose agar, the growth was moderate, compact, and green. The substratum turned amber. On sterile broad-bean stems and pods, the growth was usually less abundant. It was gray, olivaceous, or light salmon.

Conidia produced in artificial culture agreed fairly well in size with those produced on the naturally infected host, while pycnidia had great variations. The pycnidia produced on cornmeal, broad-bean-seed, and potato-dextrose agars averaged respectively 246 × 217, 307 × 286, and 246 × 241 μ.

RELATION OF TEMPERATURE AND pH TO THE FUNGUS GROWTH

In order to determine the optimal temperature for the development of mycelium, transfers were made to potato-dextrose-agar plates placed at temperatures ranging from 4° to 35° C. The fungus grew best between 20°

and 26° C., with the optimum being around 25° C. The maximum was about 32–33° C. and the minimum about 8° C.

A series of liquid media with pH ranging from 2.4 to 10 was prepared by the electrometric method. Mycelial transfers of the fungus were made to 50-ml. Erlenmeyer flasks containing 20 ml. culture fluid. The flasks were incubated at 25° C. After 9 days, the dry weight of the mycelium was determined and the average weight of 4 flask cultures representing each pH value were computed. The results indicated that a pH of 6.8 was optimal for the growth of the fungus, although moderate growth was observed up to pH 8. On the other hand, the growth rate fell rapidly towards the acid side. The fungus grew over a wide range of pH, from 3.8 to 9.

INOCULATION EXPERIMENTS

Spores from pure cultures were sprayed over the plants, in water suspension. The inoculated plants were kept in a moist chamber for at least 24 hours. The time required for the spot to appear after inoculation in the greenhouse was 4 to 7 days. The disease first appeared as tiny deep red spots which enlarged rapidly and differentiated into light centers and dark red borders. Pycnidia were soon produced at the center of the spots. Stems, pods, and petioles were readily attacked by the fungus. Inoculation of the root by mixing the mycelial growth with soil was unsuccessful.

Numerous inoculations made from 1934 to 1936 and again from 1940 to 1942 showed that the fungus was very virulent on broad bean. When too heavy a spore suspension was sprayed over young bean seedlings, they might be killed in a short time. Many full-grown plants were also killed by the fungus, either from the blight of leaves or the death of the stems.

From time to time, the fungus isolated from broad bean was inoculated to growing plants of *Phaseolus vulgaris* L., *P. angularis* L., *Glycine max* Merr., *Pisum sativum* L., *Vicia sativus* L., and *V. villosa* Roth. In all cases results were negative. Special attention was paid to the pathogenicity of this fungus to peas, which, according to Rathschlag (9), were susceptible to *A. pisi* from broad bean. However, the writer was unable to cause infection on peas with his broad-bean fungus. The fungus sometimes produced tiny red lesions on *Vicia sativus*, but the lesions remained small and never produced the fructification of the fungus.

The results of these experiments indicated that the *Ascochyta pisi* occurring on broad bean in China is highly specialized to *Vicia faba*.

OVERWINTERING OF THE FUNGUS

In the spring of 1933, diseased leaves, stems, and pods were collected and were placed in a layer about 2 inches thick over the surface of soil in large wooden boxes left outdoors. In the course of winter and spring, these boxes were taken into the laboratory and planted with broad-bean seeds coming from healthy pods. Twenty-five seeds were planted in each box and sufficient water was added daily to keep the soil moist. On January 15,

1934, spots first appeared on the lower leaves of the seedlings. Fifteen out of 125 seedlings were killed by blight of the leaves and death of the shoots at the end of February. The rest of the seedlings had lesions on the aerial parts of the plants. The experiment was repeated in the following year and the same results were obtained.

In the course of these experiments, the diseased stems and pods were brought into the laboratory from time to time and examined under the microscope. Pycnidia either with or without exuding spore masses had been observed. Conidia germinated readily in water and when healthy seedlings were inoculated with them, disease lesions appeared.

In the fall of 1933, one hundred seeds with typical lesions on their seed coats were planted in wooden boxes under moist conditions. Ninety-three seeds germinated and three of them had disease lesions on the lower stem portions. Pycnidia were observed on the cotyledons of one seedling. Forty-three seedlings produced spots on the leaves when seedlings had attained a height of 5 to 8 inches. It was, however, difficult to trace out whether the source of infection was primary or secondary. Seedlings from the healthy seed were not infected.

It is evident that *Ascochyta pisi* may persist on dead stems and pods and may also remain in the seed coat in the form of dormant mycelium until the seed germinates, when the fungus then forms pycnidia.

DISCUSSION

On account of the substantial agreement in symptomatology, and morphologic characters of the *Ascochyta* species described in this paper to that of *A. pisi* on the same host described by Sprague (12) and Rathschlag (9), it is reasonable to consider the fungus on broad bean in China as *A. pisi* Lib., the species that occurs on peas. Difference was noted, however, in pathogenicity, because the present fungus has been unable to attack *Pisum sativum* and two species of *Vicia*, while the fungus described by Sprague in North America and by Rathschlag in Germany had a wider host range. It has been shown that the *Ascochyta pisi* on *Vicia* species (12) as well as on pea and broad bean (9) are specialized to a certain degree and it is difficult to draw a specific distinction between them from the standpoint of their pathogenicity. Accordingly, a difference in host range is of little importance. Until a critical comparison between the *Ascochyta* species isolated from broad beans of various sources and extensive cross-inoculation experiments with a large number of pea varieties have been made, it is justifiable to consider the present fungus, which is highly specialized on broad bean, as a variety of *A. pisi* Lib., and the name *A. pisi* Lib. var. *fabae* Sprague is proposed for it.

SUMMARY

1. Ascochyta blight, and leaf and pod spot, of broad bean has been found in many bean-growing regions in China. Under favorable conditions, it may cause heavy losses to the crop.

2. The morphology, physiology, cultural characters, and methods of over-wintering of the fungus have been studied.

3. Inoculations show that the fungus is not able to infect peas and a few species of *Vicia*. It is thus highly specialized on broad bean.

4. From the experimental results in relation to taxonomy of the fungus the author concludes that the present fungus is a variety of the *Ascochyta pisi* that occurs on peas, and the name *A. pisi* Lib. var. *fabae* Sprague is proposed for it.

THE UNIVERSITY OF NANKING,
  NANKING, CHINA.

LITERATURE CITED

1. AGOSTINI, A. Contribuzione alle flora micologica del Senese. Arch. Bot. Sistematica **1**: 221–245. 1925.
2. CABALLERO, A. Adiciones a la microflora Española. Bol. R. Soc. Española. Hist. Nat. **28**: 421–430. 1928.
3. IDETA, A. Hand-book of the plant diseases of Japan. Ed. 4. Shokwabo, Tokyo. 1911.
4. LIBERT, M. A. Plantae cryptogamicae, quas in Arduenna collegit. Leodii, Cent. 1. 1830.
5. LUDWIG, O. Untersuchungen an *Ascochyta pisi* Lib. Beitr. Biol. der Pflanzen **16**: 464–510. 1928.
6. McDONALD, J. Annual report of the senior mycologist. Rept. Dept. Agr. Kenya (1934) **2**: 24–39. 1936.
7. NATTRASS, R. M. Annual report of plant pathologist for the year 1936. Rept. Dir. Agr. Cyprus (1936): 50–56. 1937.
8. PETHYBRIDGE, G. H. Fungus and allied diseases of crops 1925, 1926 and 1927. Gt. Brit. Min. Agr. and Fish., Misc. Publ. 70. 1929.
9. RATHSCHLAG, HEINZ. Zur Spezialisierung der auf *Vicia faba* parasitierenden *Ascochyta*. Phytopath. Zeitschr. **2**: 493–501. 1930.
10. SATTAR, A. A comparative study of the fungi associated with blight diseases of certain cultivated leguminous plants. Trans. Brit. Myc. Soc. **18**: 276–301. 1934.
11. SPEGAZZINI, C. Fungi Argentini. Anales Museo Nacional Buenos Aires **6**: 81–365. 1899.
12. SPRAGUE, RODERICK. Host range and life-history studies of some leguminous *Ascochytae*. Phytopath. **19**: 917–932. 1929.

SUMMARY

The importance of blemishes and decay of early potato tubers resulting from bacterial infection of lenticels is discussed and the various types of injuries are described and illustrated.

Bacteria were isolated from discolored and decaying lenticels of tubers from the principal regions growing early potatoes. These organisms were compared with each other and with bacteria isolated from soil about tubers and from various types of soft rot following wounds or heat injury (scald) commonly found on market potatoes. The bacteria isolated from lenticels are shown to be similar to the soft-rot organisms and equally pathogenic.

Wound inoculations with bacterial cultures from lenticels resulted in the production of decay under the same conditions that favor soft-rot development by *Erwinia carotovora*.

Lenticel infections were readily obtained in freshly harvested potatoes by immersing them for 1 minute or longer in water suspensions of bacteria isolated from lenticels.

Under controlled temperature and humidity conditions artificial inoculations did not result in decay in lenticels at 61° F. or 72° F. at a relative humidity below 94.8 per cent. At relative humidities of 98.2 per cent or above infection occurred at 61° F. but at 72° F. the decay was more rapid.

Histological studies of naturally and artificially infected lenticels in various stages of decay showed suberized parenchyma cells in the infected area. A new periderm layer, similar to that following mechanical injuries, was formed beneath the infected area under the lesions in which the decay had ceased to develop.

A study of 66 bacterial isolates from lenticels, soil, and various types of soft-rot lesions revealed that the isolates are morphologically alike and show only minor variations in their biochemical and physiological characteristics. The pathogenic isolates studied are considered to be strains of *Erwinia carotovora*.

U. S. DEPARTMENT OF AGRICULTURE

AND

UNIVERSITY OF CHICAGO.

LITERATURE CITED

1. ARTSCHWAGER, ERNST. Wound periderm formation in the potato as affected by temperature and humidity. Jour. Agr. Res. [U.S.] 35: 995–1000. 1927.
2. AYERS, S. H., P. RUPP, and W. T. JOHNSON, JR. A study of the alkali-forming bacteria found in milk. U. S. Dept. Agr. Bul. 782. 1919.
3. BARGER, W. R., G. B. RAMSEY, R. L. PERRY, and J. H. MACGILLIVRAY. Handling and shipping tests with new potatoes from Kern County, California. Calif. Agr. Expt. Sta. Bul. 664. 1942.
4. BERGEY, D. H. Bergey's Manual of determinative bacteriology. Ed. 5, 1032 pp. The Williams & Wilkins Co. Baltimore. 1939.
5. BROOKS, CHARLES, and L. McCOLLOCH. Stickiness and spotting of shelled green lima beans. U. S. Dept. Agr. Tech. Bul. 625. 1938.
6. ECKFORD, MARTHA O. Thermophilic bacteria in milk. Amer. Jour. Hyg. 7: 201–221. 1927.

7. FISHER, P. J., and JEAN E. CONN. A flagella staining technic for soil bacteria. Stain. Tech. **17**: 117–121. 1942.
8. HUCKER, G. J., and H. J. CONN. Further studies on the methods of Gram staining. N. Y. (Geneva) Agr. Exp. Sta. Tech. Bul. 128. 1927.
9. LEACH, J. G. The identity of the potato blackleg pathogene. Phytopath. **20**: 743–751. 1930.
10. ——————. Potato blackleg: The survival of the pathogene in the soil and some factors influencing infection. Phytopath. **20**: 215–228. 1930.
11. NIELSON, L. W. Solar heat in relation to bacterial soft rot of early Irish potatoes. Amer. Potato Jour. **23**: 41–57. 1946.
12. ——————, and F. A. TODD. Bacterial soft rot of Irish potatoes as influenced by sublethal temperatures. Amer. Potato Jour. **23**: 73–87. 1946.
13. RAMSEY, G. B. Fruit and vegetable diseases on the Chicago market in 1935. U. S. Dept. Agr. Plant Dis. Reptr. Suppl. **98**: 1–15. 1937.
14. ——————, J. M. LUTZ, H. O. WERNER, and A. D. EDGAR. Experiments on shipping washed early potatoes. Nebr. Agr. Exp. Sta. Bul. 364. 1944.
15. ROSE, D. H. Handling and shipping early potatoes. U. S. Dept. Agr. Circ. 744. 1946.
16. ——————, and D. F. FISHER. Desiccation effects on skinned potatoes. Amer. Potato Jour. **17**: 287–289. 1940.
17. ——————, and H. A. SCHOMER. Relation of heat and desiccation to bacterial soft rot of potatoes. Amer. Potato Jour. **21**: 149–161. 1944.
18. RUEHLE, G. D. Bacterial soft rot of potatoes in southern Florida. Fla. Agr. Exp. Sta. Tech. Bul. 348. 1940.
19. SMITH, ERWIN F. An introduction to bacterial diseases of plants. 688 pp. W. B. Saunders Co. (Philadelphia and London). 1920.
20. SOCIETY OF AMERICAN BACTERIOLOGISTS. Committee on bacteriological technique. Manual of methods for pure culture study of bacteria. Leaflet 2. Preparation of Media. 9th Ed. 1944. Leaflet 4. Staining methods. 9th Ed. 1946. Leaflet 5. Routine tests for the descriptive chart. Morphological and biochemical. 9th Ed. 1944. Leaflet 6. Further biochemical methods. 9th Ed. 1942.
21. STEVENS, N. E. A method for studying the humidity relations of fungi in culture. Phytopath. **6**: 428–432. 1916.

TUBERCULARIA CANKER AND DIEBACK OF SIBERIAN ELM (ULMUS PUMILA L.)

J . C . C A R T E R

In August, 1939, branch and trunk cankers were observed on several Siberian elms, 4 to 10 feet tall, in a commercial nursery planting. Black sporodochia of an undescribed species of *Tubercularia* were present in the diseased bark of all the cankers.

PATHOLOGY

The cankers on branches and trunks appear during April and May. They form as oval to elongate, slightly sunken areas with the long axis lengthwise of the affected tree part (Fig. 1, A). The surface of the diseased bark becomes red-brown and dotted with numerous black sporodochia (Fig. 1, A). The bark becomes brown to black internally as it dies and dries out. Cankers continue to enlarge until mid June, when callus tissue begins to develop at their margins. Cracks may form in the diseased bark, frequently at the border of the canker, as the callus tissue continues to grow over the diseased area (Fig. 1, B). The diseased bark is pushed outward, breaks and shreds, and usually peels off before the diseased area is callused over (Fig. 1, C). All cankers observed failed to enlarge in succeeding years.

The *Tubercularia* was found developing in the bark of many branches of Siberian elm affected with dieback in both nursery and ornamental plantings. It was also found developing on twigs that were dying from shading in the inner portion of the crowns of Siberian elms.

PATHOGENICITY

For pathological studies pure cultures of the fungus were grown from single spores isolated directly from sporodochia growing on diseased bark. The fungus was grown on sterilized wheat for the inoculation tests. Seven trunk and 18 branch inoculations on 21 trees were made through bark incisions on April 16, 1940.[1] Two types of bark incisions were made. One was an inverted V-shaped incision (Fig. 1, D) made with a sterile scalpel, and the other was a round hole made with a large sterile cork borer (Fig. 1, E). Wheat infested with actively growing *Tubercularia* was placed in the incisions. Some incisions were covered with parafilm (Fig. 1, F), others with Scotch tape. Sterilized wheat was placed in 5 bark incisions—2 on trunks and 3 on branches—as checks.

Infection developed from 6 of the 7 trunk inoculations and 17 of the 18 branch inoculations. No infection developed from the five check tests. In most cases, bark infection became evident within 10 days and sporodochia

[1] The date of inoculation was mistakenly stated as April 6 in the abstract published in Phytopath. 36: 395. 1946.
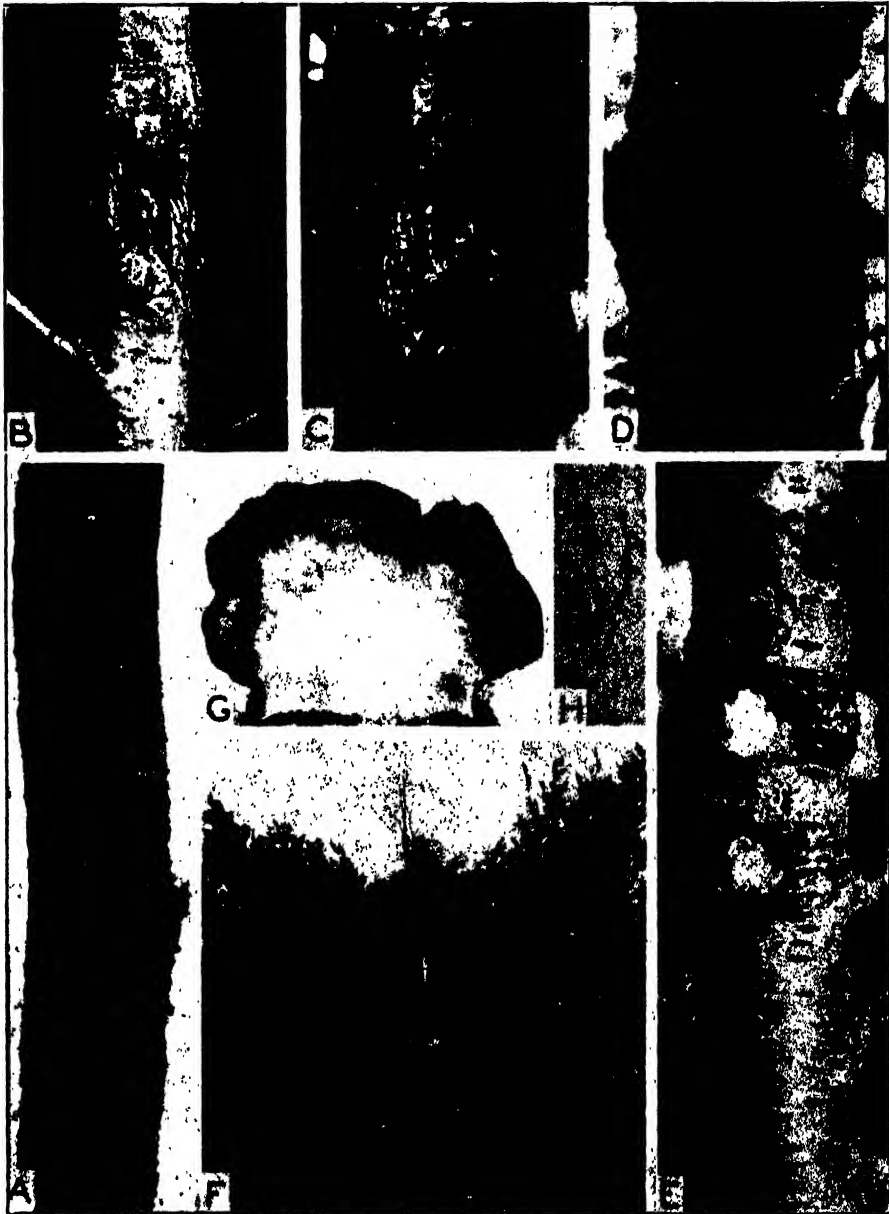
FIG. 1. Tubercularia canker and dieback of Siberian elm. A. Black, erumpent sporodochia are produced in the diseased bark. B. Trunk canker, produced by artificial inoculation, with black sporodochia on the cracked and peeling diseased bark. C. Same canker as B, callused over after 2 years. D. Trunk canker, produced by artificial inoculation, with young white to cream sporodochia on the infected bark. E. Trunk canker produced by artificial inoculation through holes made with a cork borer. F. Leader killed by infection following artificial inoculation. G. Section of an erumpent sporodochium. H. Conidia are produced acrogenously on hyaline conidiophores.

became visible in about 20 days.   First visible evidence of infection was a slight shrinking of the bark around the inoculated region.   The infected bark soon became dark gray, then red-brown; and white to cream erumpent sporodochia appeared (Fig. 1, D).   The cankers continued to enlarge until mid June when callus tissue began to develop at their margins.   Many of the inoculated branches were girdled by the infection and died distal to the inoculated regions within 35 days (Fig. 1, F).   Most of the smaller cankers were covered with callus by late August.   The larger cankers were not completely covered with callus for one to two years (Fig. 1, C).   Cankers produced by both artificial and natural inoculation failed to enlarge in succeeding years.

TAXONOMY

The sporodochia (Fig. 1, G) that develop in the diseased bark are erumpent, pulvinate, scattered to gregarious, smooth, black, horny when dry, and variable in size.   They measure up to 1.5 mm. in diameter and 0.9 mm. high. The head is concave beneath and frequently its margins rest on the host, obscuring the short stalk to which it is attached, so that the sporodochium appears to be sessile.   Sporodochia produced by artificial inoculation (Fig. 1, D) were white to cream at first but became black and horny within 5 to 6 weeks (Fig. 1, B).

Sporodochia originate in the phellogen-phelloderm region of the stem as compact masses of interwoven hyphae.   These masses continue to expand, causing a separation of the phellem and phelloderm.   The growing sporodochium ruptures and forces back the overlying phellem which forms a collar around the short sporodochial stalk (Fig. 1, G).   Hyphae continue to develop at the base of the sporodochium and are abundant in the underlying cortex and pericycle.   Scattered hyphae penetrate through the primary and secondary phloem and reach the cambium but do not invade the xylem. There appears to be little cellular disintegration of the host tissues by the invading hyphae.

Conidiophores produced on the periphery of the sporodochium head are hyaline, irregularly ramose, densely crowded, and straight to strongly curved.   They form a compact mass that covers the top of the sporodochium. Groups or bundles of conidiophores appear to branch out from a common base.   Each conidiophore has what appear to be short branches, on which the acrogenous conidia are born.   These short branches arise immediately beneath septa in the conidiophore (Fig. 1, H).   This might indicate that they are not short branches but terminal growth and that the conidiophores continue to elongate by the production of numerous branches.   The conidiophores are variable in length.

The conidia (Fig. 1, H) are hyaline, unicellular, ovoid to oblong, and occasionally somewhat allantoid.   They are produced acrogenously.   When planted on cornmeal agar, they germinated in 24 to 40 hours, each sending out a single germ tube.   Many of the germ tubes began to branch within 48 hours.   The average linear growth of the germ tubes of 6 spores was 6.1 mm.

per day over a period of 8 days. All isolates produced white colonies consisting of sparsely scattered, interwoven aerial, surface and subsurface hyphae which grow rapidly.

Owens,[2] in 1925, described a Tubercularia canker of *Ulmus pumila* L., which occurred in a nursery planting on the campus of the Oregon Agricultural College. He identified this *Tubercularia* as the imperfect stage of *Nectria cinnabarina* (Tode) Fr. The *Tubercularia* associated with the canker of Siberian elm in Illinois is not the imperfect stage of *N. cinnabarina* (Tode) Fr. It is sufficiently different morphologically from the described species of *Tubercularia* that it is considered a new species.

Tubercularia ulmea n. sp. Sporodochia erumpent, pulvinate, scattered or gregarious, black, horny when dry, up to 1.5 mm. in diameter, up to 0.9 mm. high, inhabiting diseased bark; conidiophores hyaline, irregularly ramose, crowded, straight to strongly curved, 35–87 × 1–3.5 μ, mostly 45–65 × 1.5–2.5 μ; branches short, 4–10 × 1–1.3 μ; conidia acrogenous, hyaline, continuous, ovoid to oblong, occasionally allantoid, 3.8–9.3 × 1.4–3.4 μ, mostly 4.6–6.2 × 1.5–2.3 μ.

Sporodochiis erumpentibus pulvinatis sparsis vel gregariis nigris corneis dummodo sicca usque 1.5 mm. diametris usque 0.9 mm. altis corticem aegrotam incolentibus, conidiophoris hyalinis irregulare ramosis cespitosis rectis usque valde curvatis 35–87 × 1–3.5 μ ut plurimum 45–65 × 1.5–2.5 μ, ramulis brevibus 4–10 × 1–1.3 μ, conidiis acrogenis hyalinis continuis ovoideis usque oblongis interdum allantoideis 3.8–9.3 × 1.4–3.4 μ ut plurimum 4.6–6.2 × 1.5–2.3 μ.

Type specimen: Collected by J. C. Carter, Onarga, Iroquois County, Illinois, August 16, 1939, on branches and trunks of *Ulmus pumila* L., in Illinois Natural History Survey Mycological Collection, Accession Number 29559.

SUMMARY

A canker and dieback disease of Siberian elm develops on trunks, branches, and twigs of affected trees in early spring and black sporodochia of a *Tubercularia* develop in the diseased bark. Smaller cankers are callused over by late summer; large cankers are not callused over for one or two years. Cankers were not observed to enlarge in succeeding years. This *Tubercularia* also attacks and produces black sporodochia on the twigs and small branches which are weakened or killed by shading in the interior of crowns of Siberian elm. Typical cankers and dieback were produced by artificial inoculations. The fungus is described as a new species, *Tubercularia ulmea*.

SECTION OF APPLIED BOTANY AND PLANT PATHOLOGY,

ILLINOIS NATURAL HISTORY SURVEY,

URBANA, ILLINOIS.

[2] Owens, C. E. A Tubercularia canker of Chinese elm. (Abstr.) Phytopath. 15: 729. 1925.

THE MODE OF VECTOR FEEDING AND THE TISSUES INVOLVED IN THE TRANSMISSION OF PIERCE'S DISEASE VIRUS IN GRAPE AND ALFALFA

BYRON R. HOUSTON,[1] KATHERINE ESAU,[2] AND WM. B. HEWITT[1]

INTRODUCTION

It has recently been shown (4) that the two diseases, Pierce's disease of grapes and alfalfa dwarf, are caused by the same virus. Until recently when Severin (5) reported vectors in the family Cercopidae, all of the insects found to be vectors of this virus were confined to the leafhopper sub-family Tettigoniellinae (Cicadellinae) (2, 3). This paper presents the results of a study of the feeding punctures of some of these vectors and the plant tissues involved in the transmission of the virus.

Early observations on the two leafhoppers, *Draeculacephala minerva* Ball and *Neokolla circellata* Baker, showed that they fed for long periods of time and gave off large quantities of excrement without withdrawing the mouth parts from the plant tissues. This behavior indicated that the insects were feeding on some part or parts of the vascular system. A limited number of trials showed that the excrement from a single individual female of *D. minerva* amounted to as much as 2.5 cubic centimeters in a 24-hour period. When adults of *D. minerva* were fed on excised alfalfa stems, after a dilute solution of safranin had been drawn up into the tracheary elements, it was found that some of the dye was taken out by the leafhoppers. (Sections of the stems made after the period of feeding showed the dye present only in the xylem.) The excrement from the leafhoppers was sufficiently colored by the dye to indicate that some of the material taken into their bodies was derived from tracheary elements.

FEEDING PUNCTURES

The feeding punctures of the following insect vectors were studied: adults of *Draeculacephala minerva* Ball, *Helochara sp.*, *Neokolla circellata* Baker, *Carneocephala fulgida* Nott.; nymphs of *D. minerva* and *N. circellata*.

The insects were fed on two hosts of the virus: on grapevine, *Vitis vinifera* (Emperor variety), and on alfalfa, *Medicago sativa* (California common variety). Stems, petioles, and leaf blades were examined for feeding punctures. Furthermore, *Draeculacephala minerva* was fed on healthy and dwarf alfalfa and on healthy and diseased grape. Part of the insects were previously fed on diseased plants, others were free of the virus.

The specimens bearing the feeding punctures were processed following

[1] Assistant Professor of Plant Pathology and Assistant Plant Pathologist in the Experiment Station, University of California, Davis.
[2] Associate Professor of Botany and Associate Botanist in the Experiment Station, University of California, Davis.

an ordinary paraffin method and stained according to the schedule outlined by Esau (1). The material left by the insects in the feeding punctures takes up the stains very readily and makes the punctures very conspicuous in the sections.

All the insects listed, and their nymphs, were found to be seeking the xylem tissue in the process of feeding (Fig. 1, A). The adult insects can reach the xylem in young and old plant organs. The nymphs feed on young plant organs, where the xylem is nearer the surface than in the old. In
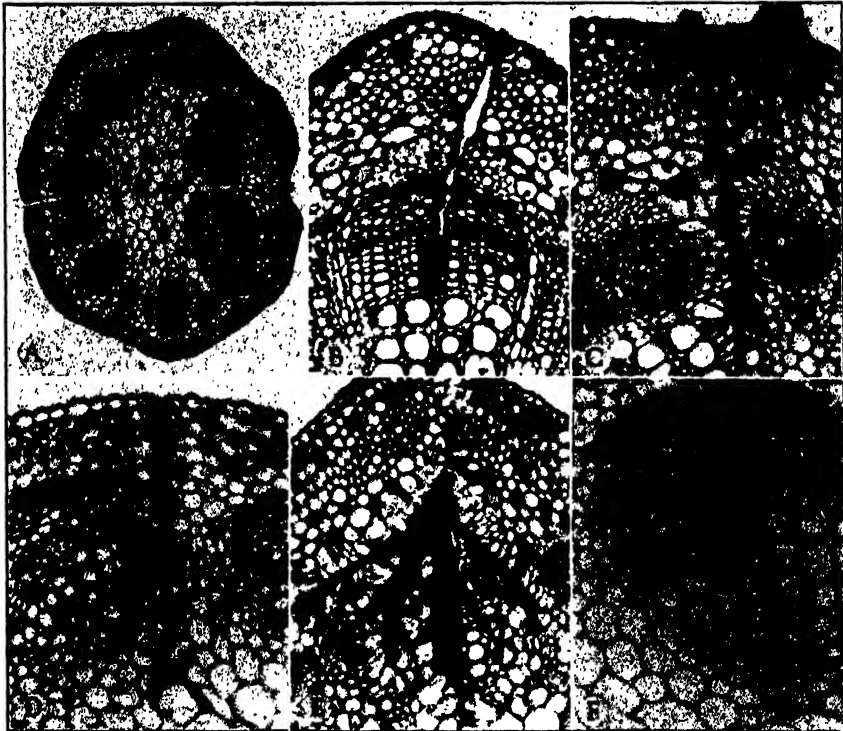
FIG. 1. Feeding punctures of *Draeculacephala minerva* upon grape (A, B, C, and E) and alfalfa (D and F). The section in A was taken from a petiole, the others from stems. The plants used for A and B were diseased, all others were healthy. A illustrates the size of the feeding puncture (to the left in the photograph) in relation to the axis as a whole. The feeding punctures in B and F passed through the phloem in reaching the xylem, those in A, C, and D did not pass through the phloem. The place of entry of the puncture in E is not shown in the photograph; only its inner, much-branched part is visible. F illustrates the penetration of feeding punctures into the lumina of tracheary elements of the xylem. A, × 25; B–F, × 145.

inserting their mouth parts into the plant organs the insects may reach the xylem by cutting in through the phloem (Fig. 1, B); or the puncture may miss the phloem and reach the xylem through the medullary ray (Fig. 1, C and D). Sometimes the insect changes the position of its mouth parts without moving to a new feeding place and leaves a much-branched feeding puncture. Figure 1, E, shows such a puncture and also indicates that the

change in position of mouth parts in this instance might have been induced by the scarcity of water-conducting elements in the region of feeding. The mouth parts of the insects either pass between or through cells. They crush some cells, come in contact with tracheary elements or penetrate into their lumina (Fig. 1, F). In feeding upon leaves, the insects manage to reach the xylem either from the upper or the lower side of the leaf blade.

In punctured stems and leaves of healthy and diseased grapevine and alfalfa, that were collected at different periods after virus-free and virus-carrying adult *Draeculacephala minerva* fed on them (collections were made on the first and up to the seventh day after feeding), 88.2 per cent of a total of 110 punctures ended in the xylem, 2.7 per cent reached the phloem, and 9.1 per cent entered parenchyma only. Of those that reached the xylem, 70.0 per cent passed through the phloem. The presence or absence of virus in the plant or in the insect appeared to have no effect upon the feeding habit of the insect.

To test further, whether or not the presence of the disease in the plant has any effect upon the mode of feeding by the insect, healthy and diseased

TABLE 1.—*The mode of feeding by Draeculacephala minerva upon healthy and diseased grape and alfalfa*

Kind of plant	Total number of punctures	Percentage of punctures		Percentage of punctures that reached xylem	
		Ending in xylem	Not ending in xylem	Through phloem	Not through phloem
Healthy grape	58	82.8	17.2	58.3	41.7
Diseased grape	172	89.0	11.0	69.9	30.1
Healthy alfalfa	159	86.8	13.2	77.5	22.5
Diseased alfalfa ..	292	93.2	6.8	87.9	12.1

grapevine and alfalfa were subjected to uniform feeding by adult virus-free *Draeculacephala minerva* (25 leafhoppers per terminal part of plant for 4 days). Grapevine petioles and alfalfa stems and petioles were examined. The results are given in table 1 and confirm the information obtained from the first lot of specimens that the majority of punctures reach the xylem and most of these pass through the phloem. The table also shows that the feeding habit of the sharpshooters was similar on diseased and healthy plants of grape and alfalfa. Among the punctures that did not reach the xylem (third data column in table 1) some ended blindly in the cortical, ray, or pith parenchyma and some (about ½ to ¼ and fewer) touched the phloem, but very few actually injured this tissue. In other words, most injury to the phloem occurred when the insects punctured this tissue in seeking the xylem.

The phloem of the grape was less frequently injured than that of alfalfa (Table 1). The cause of this is not clear. In both plants fibrous caps occur between the cortex and the functioning phloem in the somewhat older stems (Fig. 1, B and D). The insects appear to penetrate these caps without

difficulty (Fig. 1, B and F) and, on the other hand, they sometimes miss the cap in young stems, in which the fibers are without secondary walls (Fig. 1, C). Perhaps the relative size of the bundles, which are smaller in the alfalfa stem, has something to do with the higher frequency of phloem injury in alfalfa.

TISSUES INVOLVED IN TRANSMISSION OF THE VIRUS

An experiment was conducted to determine the plant tissues in which the virus after introduction by the vector would multiply and induce symptoms of the disease in potted grapevine (Emperor variety) and alfalfa
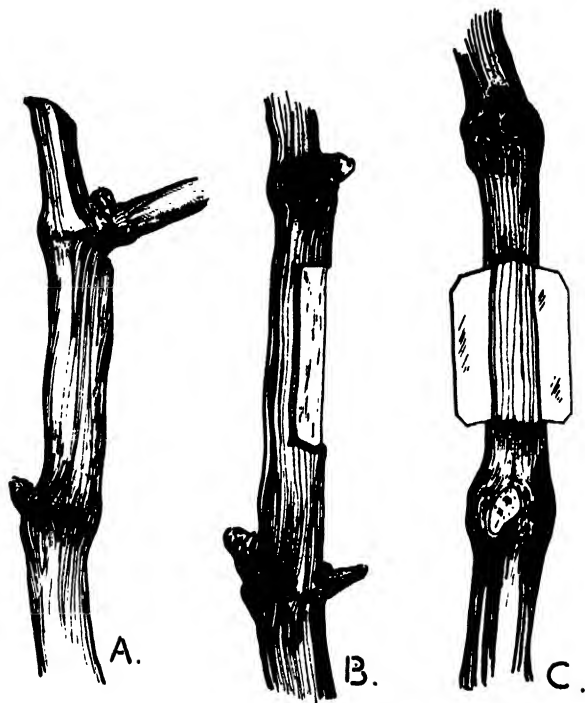
Fig. 2. Parts of grapevine stems illustrating the method of preparing them for feeding viruliferous leafhoppers upon whole stems (A); exposed xylem strip (B); cortex and phloem strip that was lifted from the xylem and separated from the latter by a plate of tin (C).

(California common variety). Viruliferous vectors were fed in the four following ways: on the entire plant including stems and leaves; confined to a three-inch portion of the lower stem; confined to an area of about two square inches of exposed xylem tissue; confined to a portion of the cortex and phloem lifted away from the xylem. The method of preparing stems for this trial is shown in figure 2. The xylem strip was prepared by removing a cortex and phloem section and covering the remainder of the stem with a double layer of tinfoil held in place with grafting wax. The exposed tissues were painted with a thin coating of Dowax[3] to prevent drying. The

[3] Commercial wax emulsion product of Dow Chemical Company.

phloem strip was prepared by making two vertical cuts about one-half inch apart and two inches long and then lifting this section away from the xylem tissue and inserting a piece of tin between the phloem and xylem.   Tinfoil was wrapped around the stem above and below the raised section.   Small cellophane cages surrounding the feeding area on the stem and cotton plugs at either end were used to confine the leafhoppers to the selected portion of the plant.   Ten viruliferous leafhoppers of either of the two species, *Draeculacephala minerva* or *Neokolla circellata*, were confined in the cages for two days.   Survival of the vectors was very good on the whole plants, whole stems, and xylem strips, but fell off very rapidly during the second day on the phloem strips.   The results of these trials are given in table 2.

Symptoms typical of the respective diseases developed only in those plants in which the vectors could reach the xylem when feeding.   The percentage of infection obtained by feeding viruliferous leafhoppers on the

TABLE 2.—*The results of feeding viruliferous vectors on various localized parts of healthy grape and alfalfa plants*

Part of plant exposed to feeding	Kind of plant	No. of plants inoculated	No. of plants diseased
Whole plant	Grape	12	11
Whole plant	Alfalfa	16	12
Whole stem	Grape	25	20
Whole stem	Alfalfa	10	8
Xylem strip	Grape	48	29
Xylem strip	Alfalfa	10	9
Phloem strip	Grape	39	0
Phloem strip*	Grape	10	0

* The strip of phloem tissue was replaced in contact with the xylem after insect feeding.

following portions of the plant were: whole plant, grape 91.6, alfalfa 75.0; whole stem, grape 80.0, alfalfa 80.0; exposed xylem strip, grape 60.4, alfalfa 90.0; phloem strip kept separated from xylem after feeding, grape 0.0; and the phloem strip replaced in contact with xylem after the feeding, grape 0.0. Three of the 39 phloem strips that were kept separated from the xylem remained alive for eight months.   Nine of the 10 phloem strips replaced in contact with the xylem (Table 2) formed a union and remained alive, but the virus did not multiply and pass from this tissue into the remainder of the plant.

As far as the writers are aware this seems to be the first record of a virus appearing to be confined in the xylem and transmitted only when the vectors could reach this tissue in the process of feeding.

MOVEMENT OF THE VIRUS IN ALFALFA STEMS

To determine the rate of movement of the virus in alfalfa stems ten infective leafhoppers of the genus *Draeculacephala* were fed for one-, two-, three-, and four-hour intervals on the basal node and internode of a number of stems.   The plants used were of a single clonal line of alfalfa known to be

very susceptible to the disease. The number of stems used in each interval of feeding was 35, 25, 15, and 25, respectively. Immediately following the feeding the stems were severed from the plant about one inch above the basal node—area of feeding—and cut into single node cuttings proceeding from the base of the stem upward. The basal cutting on which the vectors fed was then removed from the plant. All these cuttings were then rooted in sand and transplanted into sterile soil. The number of cuttings from each stem varied from four to seven, depending upon the length of the stem. The nodes were about two and one-half inches apart. Approximately 95 per cent of the cuttings survived.

After eight months the plants grown from the cuttings were examined for symptoms and the results were as given in table 3. The variation in the number of cuttings of the first four nodes was caused by the failure of a few

TABLE 3.—*Upward movement of the virus in alfalfa stems following short intervals of feeding by infective leafhoppers at the base of the stem*

Rooted node and inter-node from base of stem upward[a]	Number of cuttings[b] from plants upon which infective leafhoppers were fed for the following number of hours:							
	One hour		Two hours		Three hours		Four hours	
	Total	Diseased	Total	Diseased	Total	Diseased	Total	Diseased
First[c]	35	21	24	12	12	7	25	19
Second	35	2	24	1	12	2	22	3
Third	34	0	25	1	15	1	25	0
Fourth	30	0	22	0	13	0	22	1
Fifth	24	1	18	0	6	1	20	1
Sixth	7	0	4	0	2	0	11	0
Seventh	2	0	..		. ..		2	0

[a] The nodes were approximately two and one-half inches apart.
[b] Results were taken 8 months after the cuttings were made.
[c] The leafhoppers fed on this node and internode.

of these to root successfully. The decreasing numbers of cuttings shown for the fifth, sixth, and seventh nodes resulted from differences in the length of stems used and the failure of a few to root. It will be seen from table 3 that the cuttings made from the area of the stem on which the leafhoppers fed—the basal or first node and internode—showed a similar percentage of infection regardless of the feeding interval. The percentage of disease in these cuttings for the one-, two-, three-, and four-hour feeding intervals was 60, 50, 58, and 76, respectively. Thus, the virus survived in an average of only 61 per cent of the cuttings into which it was directly introduced. This result was somewhat similar to that of another trial in which cuttings were made from stems of diseased plants belonging to the same clonal line as those used in the experiment on virus movement. Of the 200 cuttings made four months after the plants expressed symptoms of the disease 144 or 72 per cent of them developed the disease. The virus either failed to survive in some of the cuttings or was present not in all portions of the stems.

Table 3 shows that the number and distribution of cuttings which developed the disease were about the same for all insect feeding intervals. The

data fail to show any difference in the extent of the upward movement of the virus during the different feeding intervals. In the one-hour feeding trial two of the second-node and one of the fifth-node cuttings were diseased. It appears that the virus moved upward a distance of over an inch and was present in the second node and internode within an hour after the vectors began to feed. The fact that one of the fifth-node cuttings developed the disease indicates that in this stem the virus had moved upward a distance of approximately 10 inches in an hour.

These observations suggest a rapid rate of upward movement of the virus in stems, and earlier in this paper it was shown that the vectors fed heavily upon tracheary contents and that the virus was readily transmitted to the xylem. All these observations indicate that the movement of the virus upward in a stem may be associated with the movement of water in the xylem.

SUMMARY

Studies were made on the mode of vector feeding and the tissues involved in the transmission of the Pierce's-disease virus in grape and alfalfa. Feeding punctures of the following insects were studied: adults of *Draeculacephala minerva* Ball, *Helochara sp.*, *Neokolla circellata* Baker, *Carneocephala fulgida* Nott.; nymphs of *D. minerva* and *N. circellata*. All of these insects were found to be seeking the xylem tissue in the process of feeding. In a representative lot of material using *D. minerva*, 88.2 per cent of a total of 110 punctures ended in the xylem, 2.7 per cent reached the phloem, and 9.1 per cent entered parenchyma only. Penetration of the mouth parts to reach the xylem appeared to be made at random through the phloem or medullary ray. The mouth parts either passed between or through cells including tracheary elements. The presence or absence of the virus either in the vectors or in the host plant had no effect upon their mode of feeding. The feeding of viruliferous vectors on different parts of grape and alfalfa stems showed that the virus could multiply and cause the disease only when xylem tissue could be reached by the vector during the feeding process. The rapid upward movement of the virus in alfalfa stems points to the possibility that the virus may move in the tracheary elements.

THE DIVISIONS OF PLANT PATHOLOGY AND BOTANY,
   UNIVERSITY OF CALIFORNIA,
      DAVIS, CALIFORNIA.

LITERATURE CITED

1. ESAU, KATHERINE. Anatomical and cytological studies on beet mosaic. Jour. Agr. Res. [U.S.] 69: 95–117. 1944.
2. FRAZIER, WM. NORMAN W. Phylogenetic relationship of the nine known leafhopper vectors of Pierce's disease of grape. (Abstr.) Phytopath. 34: 1000. 1944.
3. ————, and J. H. FREITAG. Ten additional vectors of the virus causing Pierce's disease of grapes. Phytopath. 36: 634–637. 1946.
4. HEWITT, WM. B., BYRON R. HOUSTON, NORMAN W. FRAZIER, and J. H. FREITAG. Leafhopper transmission of the virus causing Pierce's disease of grape and dwarf of alfalfa. Phytopath. 36: 117–128. 1946.
5. SEVERIN, HENRY H. P. Transmission of virus of Pierce's-grapevine disease and alfalfa dwarf by spittle insects, the first reported vectors in Cercopidae. (Abstr.) Phytopath. In press.

BOOK REVIEWS

GARRETT, S. D. *Root Disease Fungi*. 177 pp. 9 figs. Published by Chronica Botanica Co., Waltham, Mass. (New York City, G. E. Stechert and Co.) 1944. $4.50.

A real need for a thorough review and discussion of accumulated knowledge concerning root disease fungi has recently been satisfied by S. D. Garrett of the Rothamsted Experimental Station, England, in his book under the above title. This comprises an authoritative account of the behavior of these microorganisms in the soil and of their parasitic activities in the roots of plants, as influenced by environmental factors. It includes as well several chapters on control measures based largely on principles outlined. The treatise is the first of its kind in book form. It represents a refreshing approach to the treatment of soil-borne pathogenic fungi in that it deals with them as an ecological group rather than as a collection of more or less remotely related taxonomic entities. The result is a clear and connected exposition of a complex subject. Following an introductory chapter, dealing with the history of soil-borne plant parasitic fungi, their interrelationships with saprophytic microorganisms in the soil and general measures of root disease control, five chapters are devoted to a discussion of the influence of various factors on the parasitic behavior of root disease fungi and two to their saprophytic spread and survival in the soil. Then follow seven chapters dealing with root disease control in field, plantation, and glasshouse crops. A sixteen-page bibliography, a general index and an author index conclude the work. Errors, typographical or otherwise, e.g., "longivity" (page 2 of the contents) and "scelerotia" (page 81) are rare and there are few other detracting features, especially for a first edition. However, exception may possibly be taken to the inclusion of the smut fungi under the title chosen, to the use of the phrase "infected soil" rather than "infested soil" in several places, and to the small amount of attention given to certain control measures, e.g., the use of resistant varieties. These are minor considerations which most readers will agree are greatly overshadowed by the many virtues of the book. We are indebted to the author for bringing together in it a wealth of previously scattered information and for organizing and appraising it on the basis of his own experience. While he has naturally drawn freely on the latter he has also covered a wide range of work done by other people. In this book the author has ploughed new ground in an effective and able manner, and has made a contribution of permanent value to biology and to agriculture. A wide range of readers especially in these fields will find it an invaluable reference.—A. W. HENRY, University of Alberta, Edmonton, Alberta, Canada.

WAKSMAN, SELMAN A. *Microbial Antagonisms and Antibiotic Substances*. The Commonwealth Fund, New York. 350 pp. 34 figures. 48 tables. 1945. Price $3.75.

Dr. Waksman's extensive research in the field of soil microbiology is well known, and this book is a timely contribution to a new, fascinating, and rapidly expanding field of knowledge, and will be welcomed by both specialist and layman. There are slightly over one thousand literature references, and the subject is presented in fourteen chapters. The first three of these outline the rôle of the microbial populations in their natural habitats in reducing animal and plant wastes and the effect of their antibiotic substances on the relative ability of certain groups to survive better than others in mixed populations. In the next four chapters are reviewed the bacteria, actinomycetes, fungi, and microscopic animal forms, respectively, as producers of antibiotic substances, and the effect of these on members of their own and other groups. Perhaps the chapters most interesting are those on the chemical nature and biological properties of various antibiotics and their use in controlling bacterial diseases of man and animals. Penicillin receives merited attention, as well as a number of substances which Waksman and his colleagues have isolated, mostly from the actinomycetes. Phytopathologists will find the chapter on microbiological control of soil-borne diseases of special interest, and a convenient review of related literature. In general the conclusions of each writer are listed without critical comment, which probably is a wise course to follow at this date. A casual review of this literature reveals that phytopathologists were early pioneers in establishing that living bacteria and fungi, and substances produced by them, would definitely prevent the development of certain plant diseases caused by soil-borne and other plant pathogens. The phytopathologists will also agree with Dr. Waksman that under field conditions practical biological control of soil-borne diseases has been rather disappointing when compared with the spectacular progress made with penicillin in combating disease-producing agents in man and animals. The last chapter suggests that a really extensive search of the actinomycetes, bacteria, and fungi for new antibiotic substances of therapeutic value will be richly rewarded.—G. B. SANFORD, Pathologist in Charge, Dominion Laboratory of Plant Pathology (Science Service, Canada Department of Agriculture), University of Alberta, Edmonton.

REPORT OF THE 38TH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The American Phytopathological Society held its 38th annual meeting at the Netherland Plaza Hotel in Cincinnati, Ohio, December 28–30, 1946. Two hundred and eighty-six members from forty-one states and three foreign countries registered. One hundred and three papers were accepted by the editorial committee for presentation at the meeting. The sections and number of papers presented in each follow: physiology of fungi and antibiotics, 12; fruit diseases, 4; Dutch elm disease and other papers, 9; virus diseases, 11; field crop diseases, 12; vegetable diseases, 10; fungicides, 2 sections, 18; breeding for resistance, 8; joint meeting with the Potato Association of America, 7; and fungus diseases, 12.

Conferences included "Fungicide Colloquium," "Extension Workers," "Plant Disease Survey," "Upper Mississippi Valley Plant Pathologists," and "Tomato and Potato Late Blight Symposium."

The Phytopathologists' dinner, held in the Hall of Mirrors, Netherland Plaza, on Sunday evening, December 29, was attended by two hundred and thirty-four.

Council for 1947:

A. J. RIKER, President (1 yr.), University of Wisconsin, Madison 6, Wisconsin.

R. S. KIRBY, Vice-President (1 yr.), Pennsylvania State College, State College, Pennsylvania.

E. M. JOHNSON, Secretary (3-yr. term expires 1947), Kentucky Agricultural Experiment Station, Lexington 29, Kentucky.

M. C. RICHARDS, Treasurer, and Business Manager of PHYTOPATHOLOGY (3-yr. term expires 1949), University of New Hampshire, Durham, New Hampshire.

HELEN HART, Editor-in-Chief, PHYTOPATHOLOGY (3-yr. term expires 1948), University Farm, St. Paul 1, Minnesota.

E. E. CLAYTON, Bureau of Plant Industry, Beltsville, Maryland.

J. H. CRAIGIE, Central Experimental Farm, Ottawa, Canada.

MAX W. GARDNER, University of California, Berkeley, California.

J. H. JENSEN, North Carolina State College, Raleigh, North Carolina.

S. G. LEHMAN, North Carolina State College, Raleigh, North Carolina.

THOMAS SPROSTON, JR., University of Vermont, Burlington, Vermont.

C. M. TUCKER, Botany Department, University of Missouri, Columbia, Missouri.

Representatives:

A.A.A.S. Council. J. G. HORSFALL, R. W. GOSS.

Division of Biology and Agriculture, National Research Council. J. C. WALKER.

Board of Editors, American Journal of Botany. A. A. DUNLAP.

Standing Committees:

Donations and Legacies. A. G. NEWHALL, R. E. VAUGHN, G. F. WEBER, S. N. ZELLER, N. J. GIDDINGS, Chm.

Extension. J. O. ANDES, O. D. BURKE, O. C. BOYD, S. B. FENNE, DONALD COE, H. R. GARRISS, R. J. HASKELL, W. J. HENDERSON, T. H. KING, Chm.

Investments. MARVIN E. FOWLER, L. M. HUTCHINS, NEIL E. STEVENS, R. M. CALDWELL, M. C. RICHARDS, Chm.

Necrology. W. J. ZAUMEYER, H. D. BARKER, Chm.

Phytopathological Classics. L. C. KNORR, Editor. JOHN NIEDERHAUSER, Business Manager.

Placement. S. J. P. CHILTON, D. G. FLETCHER, M. W. GARDNER, R. J. HASKELL, L. M. MASSEY, R. S. KIRBY, Chm.

Public Relations. O. D. BURKE, L. S. HITCHNER, J. C. WALKER, H. T. COOK, J. E. LIVINGSTON, J. D. MOORE, F. J. GREANEY, J. H. JENSEN, A. G. NEWHALL, HARRY R. O'BRIEN, K. STARR CHESTER, Chm.

Regulatory Work and Foreign Plant Diseases. C. R. ORTON, R. P. WHITE, E. C. STAKMAN, Chm.

Society Organization. GEORGE ARMSTRONG, F. J. GREANEY, FREEMAN WEISS, J. B. KENDRICK, J. C. WALKER, O. C. BOYD, Chm.

Union of American Biological Societies (and Biological Abstracts). DONALD FOLSOM, L. M. MASSEY, W. C. SNYDER, FREEMAN WEISS, H. P. BARSS, G. C. KENT, Chm.; HELEN HART and E. M. JOHNSON (ex officio).

International Cooperation. H. P. BARSS, G. H. COONS, J. G. HARRAR, OTTO REINKING, J. A. STEVENSON, E. C. STAKMAN, Chm.

Special Committees:

Fungicides. M. B. MOORE, H. W. THURSTON, JR., J. D. WILSON, J. G. HORSFALL, S. E. A. McCALLAN, Chm.

Subcommittee on Seed Treatments. C. H. ARNDT, K. W. KREITLOW, J. E. MACHACEK, GEORGE SEMENIUK, R. W. LEUKEL, L. D. LEACH, W. B. ALLINGTON, C. M. HAENSELER, E. D. HANSING, M. B. MOORE, Chm.

Subcommittee on Methods. M. C. GOLDSWORTHY, C. S. HOLTON, J. G. HORSFALL, M. B. MOORE, C. F. TAYLOR, J. D. WILSON, S. E. A. McCALLAN, H. W. THURSTON, JR., Chm.

Subcommittee on Dusts and Sprays. J. M. HAMILTON, H. F. WINTER, W. H. BUCHHOLTZ, A. G. NEWHALL, J. W. HEUBERGER, A. W. DIMOCK, J. D. WILSON, Chm., and advisory members, FRANK IRONS, E. D. WITMAN, S. M. DIETZ, O. C. BOYD, H. P. BARSS, L. G. UTTER.

Subcommittee on Special Problems. J. G. LEACH, L. S. HITCHNER, DWIGHT POWELL, F. L. HOWARD, J. G. HORSFALL, Chm.

Fungus Nomenclature. C. M. TUCKER, D. S. WELCH, ERDMAN WEST, G. L. ZUNDEL, J. A. STEVENSON, Chm.

Nomenclature and Classification of Plant Viruses. C. W. BENNETT, L. M. BLACK, JAMES JOHNSON, H. H. McKINNEY, H. R. McCLARTY, FRANK McWHORTER, R. H. LARSON, FREEMAN WEISS, Chm.

Plant Disease Prevention. K. D. BUTLER, T. H. KING, R. S. KIRBY, E. C. STAKMAN, R. P. WHITE, D. G. FLETCHER, Chm.

Membership Committee. W. F. BUCHHOLTZ, W. C. SNYDER, G. C. KENT, A. A. DUNLAP, H. A. RODENHISER, E. M. JOHNSON, R. W. SAMSON, Chm.

Publication Problems. JOHN NIEDERHAUSER, W. C. SNYDER, A. J. RIKER, Chm.; HELEN HART and M. C. RICHARDS (ex officio).

National Security. M. A. McCALL, S. B. FRACKER, BERCH HENRY, W. H. WESTON, E. C. STAKMAN, H. T. COOK, H. H. THORNBERRY, Chm.

Temporary Committees for 1946:

Auditing. ERIC G. SHARVELLE, J. R. SHAY, Chm.

Resolutions. R. H. WELLMAN, R. H. LARSON, Chm.

Temporary Committees for 1947:

Sustaining Associates. R. M. CALDWELL, C. H. ARNDT, J. W. HEUBERGER, E. W. BODINE, C. E. SCOTT, A. G NEWHALL, J. J. CHRISTENSEN, Chm.

Publication of Special Material. H. P. BARSS, J. G. LEACH, W. H. TISDALE, DONALD CATION, Chm.

Report of the Secretary. On December 31, 1945, the total membership was 1100. During 1946, 72 new members were added and 64 reinstated, making a total of 1236. Fifty-seven members were suspended for non-payment of dues, 5 died, and 13 resigned. Thus the total membership on November 15, 1946, was 1161.

One hundred and forty-three names, whose applications were received after the St. Louis meeting, March 30, 1946, were elected to membership on December 30, 1946. The total membership on December 30, 1946, was 1304.

Report of the Treasurer. Statement of accounts for the year ending September 30, 1946.

Receipts:

Balance from 1945		$2378.07
Annual dues:		
1945	$ 30.00	
1946	4999.52	
1947	113.00	
1948	4.60	$5147.12
Excess illustrations		45.09
Sales		179.60
Sustaining contributors		300.00
Total receipts		5671.81
		$8049.88

Expenditures:

Member subscriptions transferred to PHYTOPATHOLOGY		
1945	$ 24.00	
1946	3998.50	
1947	90.40	
1948	3.28	$4116.18

Transferred to PHYTOPATHOLOGY for:
    Sales:

PHYTOPATHOLOGY	179.60	
Excess illustrations	45.09	224.69
Secretarial work and expenses, Office of Secretary		79.15
do , Office of President		42.38
do , Office of Treasurer		297.50
Printing and stamped envelopes		294.14
Stamps		8.00
Office supplies		1.90
Annual meeting expense		128.05
Expense of Placement Committee		21.05
Expense of Necrology Committee		1.02
Bank charges		10.72
Checks returned		20.00
Exchange charges		2.52
Refund of overpayment of dues		1.00
Miscellaneous		20.00

Total expenditures	$5268.30
Balance on hand September 30, 1946	2781.58

$8049.88

Report of the Business Manager. The total number of nonmember subscribers on November 30, 1946, was 706, representing a net gain of 152 for the year 1946. These consisted of 317 domestic, 31 Canadian, and 358 foreign subscribers. A total of 93 subscriptions were received from the U.S.S.R. Not included in the reported subscriptions for 1946 are 55 subscriptions for the current volume 36 by the American Library Association, to be held by the Society for eventual shipment to foreign countries as may be designated by that Association.

The sales of back volumes and issues during 1946 totaled $2793.74. Most of these orders were from foreign countries where PHYTOPATHOLOGY could not be sent during the war.

Statement of accounts for the year ending September 30, 1946.

Receipts:

Balance from 1945			$7318.91
Subscriptions:			
1945		$ 66.60	
1946		4200.25	
1947		295.05	
1948		3.40	$4565.30
Member subscriptions:			
1945		24.00	
1946		3998.50	
1947		90.40	
1948		3.28	4116.18
Sales of back numbers of PHYTOPATHOLOGY			2793.74
Sales of Membership List			3.50
Advertising:			
1945		377.38	
1946		829.12	1206.50
30-Year Index			162.70
Interest on Sinking Fund:			
First mortgage		21.50	
Building and Loan		50.00	
U. S. Bond Series G		25.00	96.50
Interest on current funds			124.39
Grant from Rockefeller Institute			600.00
Allowance on reprints			594.16
From authors for excess illustrations			184.04
Total receipts			14,447.01

$21,765.92

Expenditures:

Printing, distributing, and storing PHYTOPATHOLOGY:

Vol. 35, no. 9		$676.46
10		834.74
11		699.03
12		859.03
Vol. 36, no. 1 (less engraving costs)		700.55
2	do	662.74
3	do	582.79
4	do	658.05
5	do	649.76
6	do	539.64
7	do	899.85
8	do	885.71

Engravings, February–September	951.44	$9599.79	
Postage, PHYTOPATHOLOGY	565.79	$10,165.58	
Secretarial work and office expense, Editor-in-Chief		927.76	
do , Advertising Manager		59.94	
Commission for Advertising Manager, 1945		75.50	
Secretarial work for Business Manager		349.35	
Stamps		4.68	
Printing and stamped envelopes		69.52	
Office supplies		2.20	
Miscellaneous		20.16	
Postage, 30-Year Index		4.30	
Refund, subscriptions and sales		73.70	
Purchase, back volumes		31.80	
Bank charge		.62	
Total expenditures		$11,785.11	
Balance on hand:			
Checking account		4911.65	
Northwestern Federal Savings and Loan (Washington, D. C.)		5069.16	9,980.81
			$21,765.92

Sinking Fund. There was no change in the principle amount of the sinking fund during the past year, the total remaining $9676.00.

First mortgage note, at 4½ per cent interest, deposited with McLachlen Banking Corporation for collection	$ 500.00
U. S. Savings Bond, Series G, No. M1905602G, 2½ per cent	1000.00
Invested with the following:	
Columbia Permanent Building Association (accrued dividends $68.78)	568.78
District Building Loan Association (accrued dividends $202.22)	1702.22
National Permanent Building Association (accrued dividends $308.55)	2308.55
Northwestern Federal Savings and Loan Association (certificate)	2000.00
Perpetual Building Association (accrued dividend $137.56)	1137.56
Prudential Building Association (accrued dividend $32.53)	208.53
Arlington and Fairfax Building and Loan (accrued dividend $45.68)	1045.68
	$10471.32
Less interest due PHYTOPATHOLOGY	795.32
	$ 9676.00

The Lyman Memorial Fund, obtained from voluntary contributions, now totals $3310.82. This amount is invested with the Brookland Building and Loan Association at 2½ per cent. The account for 1946 is as follows:

Balance on hand, October 1, 1945	$ 3499.36
Dividends, December 31, 1945, and June 30, 1946	89.44
Voluntary contributions	115.00
	$ 3703.80
Less interest due PHYTOPATHOLOGY	392.98
	$ 3310.82

Additional Endowment:

War Savings Bond, Series F
Total, September 30, 1945 .... $ 1100.00
Contributed October 1, 1945–September 30, 1946 . . . . .   25.00
War Savings Stamps
Total, September 30, 1946 ....   7.00

$ 1132.00

The 30-Year Index. Summary of receipts and expenditures (funds deposited in PHYTOPATHOLOGY checking account) October 1, 1945, to September 30, 1946:

Balance (receipts less costs) September 30, 1945 . .. . $ 215.12
Receipts October 1, 1945, to September 30, 1946 ... .   162.70

$ 377.82
Expenses October 1, 1945, to September 30, 1946 . .. .   4.30

Balance (receipts less costs) September 30, 1946 . . . $ 373.52

Membership List Account, 1945–1946.

Balance on hand, October 1, 1945 : . ... $ 49.96
Membership lists sold 2.50

Balance on hand, September 30, 1946 . . .. $ 52.46

Report of the Auditing Committee, as of September 30, 1946. We have examined the books of the Treasurer of The American Phytopathological Society and of the Business Manager of PHYTOPATHOLOGY for the period October 1, 1945, to September 30, 1946. We found all receipts and expenditures and all funds of the Society and of PHYTOPATHOLOGY lucidly and accurately recorded. Miss Melba K. House is to be highly commended for her excellent performance in maintaining these records.

Signed: J. R. SHAY, *Chairman*
E. G. SHARVELLE

C. P. A. Report on books of Treasurer and Business Manager for October 1, 1946, to January 21, 1947. Pursuant to the request of Dr. Ralph M. Caldwell, I have examined the records pertaining to cash receipts and disbursements of The American Phytopathological Society and PHYTOPATHOLOGY, of which he is Treasurer and Business Manager, respectively, for the period from October 1, 1946, to January 21, 1947.

Verification of cash receipts extended only to substantiating that all receipts which were entered in the cash books were deposited in the designated depository. All cash disbursements were substantiated by receipted vouchers. The cash balances at the end of the period were verified by confirmations received from the respective depositories. Assets of The American Phytopathological Society set aside for specific purposes consisting of government securities and deposits in building and loan associations were also verified.

Based on the above examination, in my opinion, the statements of The American Phytopathological Society and PHYTOPATHOLOGY correctly set forth the receipts and disbursements for the period reviewed.

February 10, 1947

C. D. D'AOUST
Certified Public Accountant
Lafayette, Indiana

Report of the Advertising Manager. PHYTOPATHOLOGY carried a total of 108 paid advertisements during the year 1946. There were 63 full-page, 33 half-page, and 12 quarter-page advertisements.

The gross income from advertising was $1,421.50. The net income to the Society will appear in the Treasurer's report. Advertising agencies are allowed a 15 per cent commission and a 2 per cent discount is permitted when bills are paid within 10 days. This accounts for the difference between gross and net income.

In addition to paid advertisements, the Journal has carried some announcements and notices for the Society in the advertising section.

Report of the Editor-in-Chief. The 1059 pages of volume 36 of PHYTOPATHOLOGY were used by 278 authors in the publication of 89 long articles, 37 phytopathological notes, 122 abstracts, 7 biographical sketches, and 1 book review. Three reports and three announcements were published for The American Phytopathological Society. The

volume contained 7 portraits and 221 illustrations for scientific articles and notes. Of the 207 tables in the volume, 190 each required less than a full page, 12 required full pages, and 5 required from one and one-half to 7 pages each. The November issue contained the manual "Preparation of manuscripts for PHYTOPATHOLOGY," written by Dr. A. J. Riker and authorized by the Society at its annual meeting in December, 1944.

On December 1, 1946, there were 50 papers on hand: 23 had been accepted for publication and 15 of these were in press; 16 were being revised by the authors; and 11 were under consideration by the editors. Between March 1 and December 1, 1946, 12 papers were withdrawn or rejected.

The time between submittal of a paper and its acceptance varies from 6 weeks to 10 months, depending on the clarity of the paper, the criticisms of the reviewers, and the advisability of revision. The time between acceptance of a manuscript and its appearance in the Journal varies from 4 to 6 months. From 1 to 2 months are required for marking copy and for printer's composition work; approximately 1 month is needed for correction of galley proofs and submittal to authors; and approximately 2 months elapse between placement in a monthly number of the Journal and the issuance of the number.

Again we are indebted to the Rockefeller Institute for financial support of the journal.

It is a pleasure to acknowledge the editorial and clerical assistance of Frances Cooper, the work of J. M. Daly in preparation of the index and in proof-reading, and the excellent cooperation of editors and associate editors.

The editors have contributed considerable time and effort in reviewing manuscripts, and they have attempted to judge each manuscript on its merits and its present and future value. Judgment has been difficult at times because several manuscripts have dealt with scientific research in fields bordering on plant pathology.

Report of Representative in the Division of Biology and Agriculture, National Research Council, for 1946. Since our annual meeting in March 1946, several matters of importance to the Society have come before the Division:

(1) The proposal for an Institute of Biologists to represent Biology as a whole in National Affairs was presented by D. W. Bronk (now Secretary of the National Research Council). An informal gathering of representatives of various societies to discuss further action was suggested. H. P. Barss, our representative of the Union of Biological Societies, was asked to represent us, and J. G. Leach was asked to serve as alternate. Progress to date has been slow. No meeting has yet been called. Dr. Griggs has been asked to solicit opinions of biologists individually. This is a matter which the Council should discuss and provide for general expression of opinion of members during the Cincinnati meeting.

(2) The UNESCO has now been joined by the United States. A Preparatory Commission is drawing up plans for the ultimate organization. This Commission consists of nine divisions. The Division of Natural Science is the one with which our Science will be concerned. Ultimately the President will appoint five delegates to UNESCO and a 100-member national commission to advise them. The development of organization and policies of UNESCO is likely to be slow at times and precipitate at other times. It is well that our Society designate some member, preferably a resident of Washington, to keep in touch with the Division of Biology and the Division of Foreign Relations on UNESCO matters. This is the time when suggestions from the Society as to how UNESCO can be useful to our field of science will be welcome. All matters of international cooperation are involved, such as: revival of International Unions; plans for international meetings or congresses; exchange of students and personnel, etc. Time should be taken to discuss this matter at Cincinnati, and some expression of Society action sought.

(3) The Office of Scientific Personnel of NRC is active under the direction of M. H. Trytten. Availability of Phytopathological Personnel reported to our Society by K. Starr Chester February 25, 1946, was made available to him to incorporate in a report for Biology as a whole.

(4) The NRC Committee on Genetics of Microorganisms, of which Rodenhiser, Riker, Stakman, and McKinney are members, is planning a symposium in connection with the AAAS 1947 summer meeting.

(5) A new interdivisional committee known as the Chemical-Biological Coordination Center has been organized with the objective of correlating chemical structure and biological activity by cataloguing chemical compounds in terms of their structure, physical properties, and action upon plant and animal organisms. A biologic code is being worked out with breakdown in terms of various fields including plant pathology. Members in fungicide and antibiotic research may find this a useful source of information, and any interested may direct inquiries to Dr. W. R. Kirner, Director, Chemical-Biological Coordination Center, National Research Center, Washington, D. C.

Report of the Necrology Committee. The following members died during 1946:

JOHN L. RUE	February 8
GROVER H. BURNETT	June 16
R. A. HARPER	May 12
G. A. SCOTT	February 2
F. C. STEWART	April 24

Report of the Manager of Phytopathological Classics. Report for the fiscal year beginning October 1, 1945 and ending September 30, 1946:

Classic No. 1:	On hand, October 1, 1945	6	
	Sold during year	6	$ 3.00
	On hand, September 30, 1946	0	
Classic No. 2:	On hand, October 1, 1945	208	
	Sold during year	30	15.00
	On hand, September 30, 1946	178	
Classic No. 3:	On hand, October 1, 1945	298	
	Sold during year	33	16.50
	On hand, September 30, 1946	265	
Classic No. 4:	On hand, October 1, 1945	359	
	Sold during year	31	23.25
	On hand, September 30, 1946	328	
Classic No. 5:	On hand, October 1, 1945	594	
	Sold during year	33	41.25
	On hand, September 30, 1946	561	
Classic No. 6:	On hand, October 1, 1945	681	
	Sold during year	35	26.25
	On hand, September 30, 1946	646	
Classic No. 7:	On hand, October 1, 1945	702	
	Sold during year	38	28.50
	On hand, September 30, 1946	664	

	$153.75
Value of books sent out (fiscal year 1945–1946)	$153.75
Money received on orders of previous year	5.50
	$159.25
Money received during fiscal year 1945–1946	$157.25
Due on account	2.00
	$159.25

Assets:

Cash balance on hand, October 1, 1945	$497.53
Receipts during year	157.25
	$654.78

Liabilities:

Stationery (overprinting)	12.50
Balance on hand, September 30, 1946	$641.53
Total due on account, September 30, 1946	$ 16.75

Report of the Editor of Phytopathological Classics. The eighth number in the series PHYTOPATHOLOGICAL CLASSICS will bear the title page: Observations, Botanical and Physiological on the Potato Murrain, by M. J. Berkeley, together with selections from Berkeley's Vegetable Pathology made by the Plant Pathology Committee of the British Mycological Society.

In addition to material indicated in the title, there is to be a 3500-word biography of Berkeley written by Dr. J. Ramsbottom of the British Mycological Society.

As a token of appreciation for the efforts of our British colleagues in the preparation of this latest PHYTOPATHOLOGICAL CLASSIC it is hoped that publication can be timed to coincide with the Jubilee Year of the British Mycological Society.

Negotiations are currently under way for the publication of Number 8 with the printer, Stone Printing Company of Lansing, Michigan, the firm submitting low bid. The cost of printing will be paid out of profits from the sale of previous issues, thereby maintaining the self-sufficient basis of managing CLASSICS.

Manuscripts are being considered for the issue to follow the one on Berkeley and further suggestions are being solicited. Qualified opinion on the merits of Tozzetti's ALIMURGIA has been surveyed, and the editor is being urged to undertake the publication of the work, which Dr. J. C. Walker deems "one of the best contributions in the

period from 1750 to 1800'' and in which Count Re sees historically the first real suggestion that plant diseases are caused by specific organisms. Other proposals for number 9 include (1) a translation of Dr. M. Navaschin's paper on *Sclerotinia betulae* (a project contemplated by the late Professor H. H. Whetzel), (2) a review of Russian phytopathological research, and (3) selections from the works of Erwin F. Smith covering in particular detail the Smith-Fischer controversy.

Report of the Placement Committee. This was the busiest year on record for the Placement Committee. During 1946, 50 plant pathologists had applications filed with the Committee which were sent to prospective employers. A total of 209 individual applications were sent to 40 prospective employers who requested them.

Incomplete reports show that at least seven plant pathologists obtained positions through the efforts of the Placement Committee.

Report of the Public Relations Committee for 1946. The procedure adopted by the Committee in 1945 has been continued. During the 8-month period since the last report, sixteen ''Ideas'' for feature stories on plant pathology have been furnished editors of national magazines. Eleven of these have developed into published stories, and publication of two others is now being arranged. One story fully illustrated in color has appeared in ''Successful Farming,'' and arrangements have been completed for an all-color photographic essay of unusual type in ''Better Homes and Gardens.'' The chief problem has been to furnish enough material to meet the requests of magazine editors.

The work of the Committee has proceeded at no expense to the Society, secretarial facilities and communications having been donated.

The Committee has been aided in its duties by the independent efforts or special assistance of other members of the Society, and it takes this opportunity of thanking them and of renewing its request for additional ''Ideas'' from any Society member.

Report of the Committee on Biological Abstracts and the Union of Biological Societies. The Committee has renewed consideration of several subjects which could not be furthered during the war, and has considered several new projects. It is recommended that The American Phytopathological Society join with other biological societies in making an annual contribution to the support of Biological Abstracts.

It is further recommended that The American Phytopathological Society petition the UNESCO and the FAO, and the National Science Foundation if it be established, to designate Biological Abstracts as their official abstracting service in biology in the United States and to provide the needed financial support.

It is also recommended that a committee of the Society be appointed to investigate the needs and desirability of some type of Annual Review of Phytopathology.

Arrangements are under way to institute the publication in Section D, Abstracts of Plant Sciences, of Biological Abstracts, of abstracts of movie films and other visual aids as they become available.

Report of the Committee on Resolutions. Be it resolved that The American Phytopathological Society express its grateful appreciation to the following for their contributions to the success of the 38th annual meeting:

Stephen Diachun, T. H. King, O. T. Wilson, J. D. Moore, and Paul E. Tilford for so capably handling all local arrangements in connection with the sectional meetings.

W. D. Valleau and Stephen Diachun for arranging all papers into sections.

The University of Wisconsin, Ohio State University, and Purdue University for projection equipment.

The management of Hotel Netherland Plaza for furnishing facilities for the meeting. Special thanks are due Miss Mary Hesse, Mr. Otto Hicker, and Mr. Oscar Kline, of the hotel staff, for very splendid cooperation.

The Cincinnati papers, The Times Star, The Post, and The Enquirer, for news coverage.

The Cincinnati Convention Bureau for handling registration and assisting in the sale of banquet tickets.

Be it separately resolved that the Society express its appreciation for the invaluable services of Dr. E. M. Caldwell during his term as Treasurer and as Business Manager of PHYTOPATHOLOGY.

Respectfully submitted,
R. H. WELLMAN
B. H. LARSON

Elections and Appointments. A committee from the Council opened and counted the ballots, results of which were announced to the Society at the banquet the evening of December 30: A. J. Riker, President; R. S. Kirby, Vice-President; J. H. Jensen, Councilor-at-large.

The Council recommended and the Society approved the appointment of H. A. Rodenhiser as Editor for a three-year term through 1949; F. L. Drayton, S. M. Zeller, M. F. Kernkamp, and G. M. Armstrong as Associate Editors for three years through 1949; M. C. Richards, Treasurer, and Business Manager of PHYTOPATHOLOGY for a three-year term through 1949; L. J. Alexander, Advertising Manager of PHYTOPATHOLOGY for 1947; L. C. Knoor, Editor of PHYTOPATHOLOGICAL CLASSICS for 1947; John Neiderhauser, Business Manager of PHYTOPATHOLOGICAL CLASSICS for 1947; R. W. Goss, Representative on A.A.A.S. Council for a two-year term through 1948.

Representatives of the Society and changes in committee personnel are given in the previous pages of this report.

One hundred and forty-three applicants were elected to membership in The American Phytopathological Society.

Reports of Officers, Representatives, and Standing Committees are published on the previous pages. According to action of the Society at the Philadelphia meeting, reports of Special and Temporary Committees are not to be published in the annual report. All committee reports submitted were considered by the Council. Those recommended for approval by the Council were accepted by the Society.

The Society approved the following recommendations by the Council:

1. That the incoming Treasurer be paid his expenses from Cincinnati, Ohio, to Lafayette, Indiana, and return in order to confer with the outgoing Treasurer relative to his duties.

2. That the advertising rates in PHYTOPATHOLOGY be raised according to a schedule submitted by the new Advertising Manager.

3. That fifty copies of one selected issue of PHYTOPATHOLOGY be made available to the Advertising Manager for use in soliciting advertising.

4. That the Society expresses its interest in bringing biologists together for more effective cooperation, and recommends that J. C. Walker, its representative in the Division of Biology and Agriculture, National Research Council, represent the Society in discussions relative to an Organization of Biologists; that he be given the privilege of selecting other members to assist him.

5. That annual dues be raised to $6.00 beginning January 1, 1948.

6. That annual subscriptions to PHYTOPATHOLOGY be increased to $7.50 beginning January 1, 1948.

7. That the sections relative to life membership in both the proposed Constitution and in the Standing Rules be deleted.

8. That the Committee on Terminology (Nomenclature) of Immunology and Use of Technical Words be abolished.

9. That an over-all Committee on Fungicides be established composed of the Special Committees of the Society dealing with fungicides together with the chairman of the Subcommittee on Fungicides of the War Committee; that the above committees become subcommittees of the over-all committee; that the new committee rotate the chairmanship annually among the various subcommittee chairmen; that the new over-all fungicide committee be empowered to add to its membership and set up subcommittees as it sees fit; that S. E. A. McCallan be designated as chairman of the over-all committee for 1947.

10. That the Subcommittee on Coordination in Cereal and Vegetable Seed Treatment Research be changed to the Subcommittee on Seed Treatment; that the Subcommittee on Standardization of Fungicidal Tests be changed to the Subcommittee on Methods; that the Subcommittee on Coordination of Field Tests with New Fungicidal Dusts and Sprays be changed to the Subcommittee on Dusts and Sprays; that the Subcommittee on Fungicides of the War Committee be changed to Subcommittee on Special Problems.

11. The appointment of R. W. Goss as the Society's representative on the A.A.A.S. Council for 1947-1948.

12. That the final report of the Executive Committee of the War Committee be accepted and the Committee dissolved.

13. That both copies of the War Committee report be given permanent bindings; that one copy be deposited in the Library of Iowa State College along with the Committee report of World War I; that the second copy be filed with the Secretary.

14. That the Special Committee on Reorganization of International Cooperation be made a permanent committee to be known as the Committee on International Cooperation.

15. That the manuscript entitled "The test tube dilution technique for use with the slide germination method of evaluating protectant fungicides" be published in PHYTOPATHOLOGY, subject to the usual editing.

16. That a new Special Committee to be known as the Committee on National Security be formed.

17. That, at his request, the books of the retiring Treasurer, and Business Manager of PHYTOPATHOLOGY be audited by a certified public accountant at the Society's expense.

18. That the expense of the new Treasurer, and Business Manager of PHYTO-PATHOLOGY, be paid to visit the Burlington Free Press and Science Press in order to consult with them on publication problems. ˙

19. That the Society give $100 to Biological Abstracts.

20. That the following resolutions be adopted: (a) "The American Phytopathological Society recognizes the outstanding services rendered by Biological Abstracts in the advancement of scientific research and education and hereby expresses the hope that UNESCO, FAO, and such agencies as the proposed National Research Foundation may recognize the usefulness of Biological Abstracts, may avail themselves of its services, and may give encouragement to its work as an instrument of scientific progress"; (b) "In view of the heavy losses sustained by American agriculture from sweeping outbreaks of plant diseases, The American Phytopathological Society considers that one of the most important services needed by American farmers is a more effective reporting and fore-casting service, and a vigorous program of research basic to such a service. The support of all those responsible for the administration of agricultural research is solicited toward this end"; (c) "Be it resolved that The American Phytopathological Society recommend to the 80th Congress the prompt passage of insecticide and fungicide legislation to replace the outmoded Federal Insecticide Act of 1910; be it resolved that The American Phytopathological Society go on record and advocate the passage by the several states of the Model State Insecticide-Fungicide Act where such new legislation is deemed necessary or where existing state laws are being modified to obtain uniformity"; (d) "In publication 16 of the International Crop Improvement Association, entitled 'Minimum Seed Certification Standards,' it is noted that minimum tolerances have been recommended for certain seed-borne plant diseases. This Society wishes to commend the Association for making this start, and to urge continued effort toward the improvement of these tolerances and their extension to other diseases and to other crops. The American Phytopathological Society offers its cooperation in helping this worthy cause to the end that seed-borne diseases may be brought under better control through the general use of disease-free, certified seed"; (e) "Many projects in plant pathology, entomology, genetics, biochemistry, plant physiology, ecology, and other basic sciences cannot be organized exclusively on a crop basis, nor can such projects be formulated around a single field of plant science. To adhere to the crop basis concept eliminates from consideration much of the most fundamentally important work in the plant sciences. For this reason it is respectfully suggested that when future projects of the Hope-Flannagan type are planned, provision be made for consideration of some projects on a more comprehensive science subject basis"; (f) "That an attempt be made to establish the science of Plant Pathology as a distinct science unit to be recognized as such in the biological science group; that scientists trained as plant pathologists make greater progress when allowed to operate as a group or department rather than divided into various related groups or departments."

20. That the President shall appoint alternates for Councilors-at-large who are unable to attend official meetings; that this directive be added to the new Constitution.

21. That the Proposed Constitution, prepared by the Committee on Society Organization, be adopted with the following changes: Article II, Section 1, delete Life Membership. Section 2,d Life Membership, delete. Article IV, add Section 7, The President shall appoint alternates for Councilors-at-large who are unable to attend official council meetings. Standing Rules. 1. Change (b) to read, Members whose dues are in arrears one month shall be dropped from the rolls. Members in arrears may be reinstated, without the formality of reelection, by payment of current dues. 2. (c) Line 2, change $6.00 to $7.50. Line 3, after Canada, change $6.25 to $7.75, and after other countries, change $6.50 to $8.00. Line 4, change 60 cents to 75 cents. (f) Line 1, delete the word all. 7. (c) Line 1, insert Summaries of, after Publication. (d) Delete. 8. (e) Line 1, insert summaries of, after publication of. 9. Delete.

22. That The American Phytopathological Society meet with A.A.A.S. unless The Botanical Society of America should meet elsewhere, in which case the Program Committee is empowered to make arrangements to meet with The Botanical Society of America if it considers such a meeting desirable.

REVISED CONSTITUTION OF THE AMERICAN PHYTOPATHO-LOGICAL SOCIETY

(Ratified at the 38th Annual Meeting of the Society, Cincinnati, Ohio, Dec. 30, 1946)

ARTICLE I—NAME AND OBJECTIVES

The name of this Society shall be The American Phytopathological Society. Its objects are to promote: (1) the increase and diffusion of all aspects of knowledge relating to plant diseases and their control, (2) effective working relations between persons professionally engaged in this field of study, and (3) coordination of various interests and services pertinent thereto.

ARTICLE II—MEMBERSHIP

Section 1. The Society shall consist of Charter Members, Annual Members, Patrons, and Sustaining Associates.

Section 2. (a). *Charter Members.* The Charter Members are the one hundred thirty persons who accepted the invitation of the Organization Committee of October 25, 1909, to form the Society.

(b). *Annual Members.* All persons interested in the study of phytopathology, including the practical control of plant diseases, shall be eligible to membership. Members may be elected at any regular meeting of the Society or by the Council in the interim. Applications for membership shall be endorsed by at least one member of the Society. Annual members shall pay such annual dues as are prescribed in the Standing Rules.

(c). *Patrons.* Any person making a payment to the Society of such amount as may be prescribed in the Standing Rules shall be designated a Patron and, upon election to membership, shall have all the privileges of an Annual Member.

(d). *Sustaining Associates.* Any firm making an annual contribution to the Society of such amount as prescribed in the Standing Rules shall be designated a Sustaining Associate, and shall receive the official journal without charge.

ARTICLE III—OFFICERS

Section 1. The officers of the Society shall be a President, a Vice President, a Secretary, and a Treasurer.

Section 2. The duties of the officers shall be those customarily pertaining to these offices.

ARTICLE IV—COUNCIL

Section 1. The government of the Society, and its corporate authority as defined in the Society's Articles of Incorporation, shall be vested in a Council which shall consist of the contemporary officers, the retiring President, and the Editor-in-Chief and the Business Manager of the journal PHYTOPATHOLOGY, a Councilor from each unit of the Society that is organized as a Division, and two Councilors-at-Large.

Section 2. The President shall call meetings of the Council at his discretion or upon the written request of three of its members. He, or in his absence, the Vice President, shall preside at meetings of the Council.

Section 3. The presence of a majority of the Council shall constitute a quorum for the transaction of business.

Section 4. All actions of the Council or officers must be authorized or approved by the Society at the annual meeting except as specified in Article VII, Section 3.

ARTICLE V—SELECTION OF OFFICERS AND COUNCILORS

Section 1. The President, Vice President, and Councilors-at-Large shall be elected by ballot. The Secretary shall send a nomination ballot for these offices to all members of the Society in time to allow nominations to be returned not less than two months before the date of the annual meeting. The Council shall designate the nominees for any office when such nominations are wanting, or are tied. The names of the three persons who receive the highest number of nominating votes for each office shall be placed on a final ballot, which shall be sent to each resident member not less than one month before the annual meeting. If the same person should receive sufficient ballots to qualify for nomination to more than one office, his name shall be placed on the final ballot as a candidate for only the higher office as listed in Article III, Section 1. These ballots shall be returned to the Secretary and shall be canvassed by the Council. A plurality vote shall elect.

Section 2. The Secretary and the Treasurer shall be appointed by the Council, ordinarily for terms of three years, which shall not expire concurrently, and the Council may adjust the term or the date of assuming office to avoid this contingency.

Section 3. The President and the Vice President shall assume office upon the final adjournment of the annual meeting at which they are elected. They shall not be eligible to immediate reelection to the same offices.

Section 4. The terms of the Councilors-at-Large shall be two years beginning with the final adjournment of the annual meeting at which they are elected, or until their successors are elected.

Section 5. Division Councilors shall be elected by their respective Divisions in such manner and for such terms as the Divisions may prescribe.

Section 6. The Council shall fill by appointment any vacancy, except that of a Division Councilor, occurring within the prescribed term, such appointment to continue for the unexpired balance.

Section 7. The President shall appoint Alternates-at-Large for the Councilors-at-Large who are unable to attend the official Council meetings.

ARTICLE VI—SOCIETY JOURNAL AND EDITORIAL BOARD

Section 1. The official publication of the Society shall be the journal PHYTO-PATHOLOGY.

Section 2. The Council shall appoint an Editor-in-Chief and a Business Manager of the Journal for terms of three years not expiring concurrently, and also an Editorial Board as prescribed in the Standing Rules.

Section 3. The Council may authorize the Editor-in-Chief and the Business Manager to employ such assistants as may be necessary for the proper conduct of their work.

ARTICLE VII—COMMITTEES AND CONDUCT OF SOCIETY BUSINESS

Section 1. The Council shall appoint such standing and special committees as may be appropriate for conducting the business of the Society.

Section 2. (*a*). *Standing Committees.* Committees whose functions include the general policies and internal relations of the Society, and its relations with other organizations, shall be known as Standing Committees. They shall have a revolving membership and shall submit an annual report to the Council at the Society's annual meeting.

(*b*). *Special Committees.* Committees whose function is to deal with special subjects of concern to the Society shall be designated Special Committees. Each special committee shall be continued for such period as in the judgment of the Council may be necessary for the accomplishment of its purpose. Interim reports may be requested and a final report shall be made to the Council at the close of this period.

(*c*). *Temporary Committees.* Committees for the accomplishment of a specific purpose, limited in scope and time, may be appointed by the President to serve during his term and to make such reports to the Council as he may direct.

Section 3. In the interim between regular meetings of the Society the Council may undertake and carry out such actions as it deems advisable or necessary, including the reference of particular questions to the Society members by mail or by notices in the official journal, subject to approval by the Society at its next meeting.

ARTICLE VIII—FUNDS

The control of funds of the Society received from dues, subscriptions to the Journal, gifts, bequests and endowments shall be vested in the Council to administer through the appropriate fiscal officers and committees. An audit of the receipts and disbursements shall be made annually by a temporary committee, or at the discretion of the Council by a certified public accountant. The reports of the Treasurer and the Business Manager, together with that of the auditing committee, shall be published annually in the official journal.

ARTICLE IX—MEETINGS

A general meeting of the Society shall be held each year, unless prevented by a national emergency, at such place and time as the Council may direct. Special or local meetings for the presentation of scientific papers or demonstrations of experimental results may be arranged at the discretion of the Council.

ARTICLE X—DIVISIONS

Section 1. Branch organizations or units within the Society, known as Divisions, may be established within the geographical area of North America, provided that a formal application setting forth the reasons for such establishment is made to, and approved by, the Society.

Section 2. The organization and the operations of Divisions shall be governed by the specifications relative to Divisions in the Standing Rules.

ARTICLE XI—AFFILIATED SOCIETIES

For the purpose of promoting international collaboration between professional organizations in the field of plant pathology, the Council of The American Phytopathological Society is authorized to establish affiliated-relations with other phytopathological societies in accordance with the provisions pertaining thereto in the Standing Rules.

ARTICLE XII—RATIFICATION AND AMENDMENT

Section 1. This Constitution shall become effective upon its ratification in accordance with the procedure for amending the original Constitution of the Society. It shall supersede the original Constitution and all amendments thereto.

Section 2. This Constitution may be amended at any annual meeting of the Society provided that any proposed amendment be approved by the Council and be published in the official journal, or otherwise communicated to all resident members, at least one month before the annual meeting, and that it receive the affirmative votes of three fourths of the members voting at a regularly scheduled business session.

STANDING RULES

1. DUES, ARREARS, REINSTATEMENT

(a). The dues for all annual members as defined in Section 2(b), Article II of the Constitution, including subscription to PHYTOPATHOLOGY, shall be $6 a year, payable by February 1. The Business Manager is authorized to discontinue sending the journal to members whose dues have not been paid by this date.

(b). Members whose dues are in arrears for 1 month shall be dropped from the rolls. Members in arrears may be reinstated, without the formality of reelection, by payment of current dues.

(c). The contribution of a Patron shall be one thousand dollars, payable in one sum.

(d). The annual contribution of a Sustaining Associate shall be at least one hundred dollars, and a list of Sustaining Associates shall be published in each issue of the Journal.

(e). The payments received from Patrons and Sustaining Associates shall be credited to the current funds of the Society. These funds shall be transferable to the account of PHYTOPATHOLOGY, or may be used for other purposes authorized by the Council.

2. PHYTOPATHOLOGY

(a). *Editorial Board.* The editorial policy of the official journal, PHYTOPATHOLOGY, shall be vested in an Editorial Board consisting of an Editor-in-Chief, three Editors, and twelve Associate Editors; the business control shall be vested in a Business Manager and an Advertising (or Assistant Business) Manager.

(b). *Selection of Board Members.* This Board, aside from the Editor-in-Chief and the Business Manager, the manner of whose appointment is prescribed in Article VI, Section 2 of the Constitution, shall be selected from the membership by the Council in consultation with the Editor-in-Chief, and approved by the Society.

(c). *Terms of Office.* The term of each member of the Board shall be three years, provided that the terms of the three Editors shall not be concurrent, and that four Associate Editors shall be appointed each year. The Council is authorized to make any adjustments in these terms of office necessary to effect non-concurrence.

(d). *Compensation of Advertising Manager.* The Advertising Manager may receive compensation for his services on a commission basis as determined by the Council.

(e). *Subscriptions and back numbers.* Subscriptions to PHYTOPATHOLOGY for institutions and non-members shall be $7.50 per year in the United States and dependencies; Canada, $7.75; other countries, $8.00. The price of current single numbers shall be 75 cents. The sale and price of back volumes or numbers shall be determined by the Business Manager with the approval of the Council. Requests to supply lost copies of the journal without charge must be made within sixty days from date of issue.

(f). *Income from advertising.* Income received from the sale of advertising space in PHYTOPATHOLOGY shall be devoted to the production of the journal.

3. PROGRAM COMMITTEE

The program for the annual meeting shall be in charge of a temporary committee consisting of the President, Vice President, Secretary, Editor-in-Chief, and such addi-

tional members as the President may select. This committee shall have full authority over the scheduling of sessions and demonstrations, and the allocation of papers.

4. EXPENSES OF OFFICERS AT ANNUAL MEETING

The Secretary, the Treasurer, and the President, or in his place the Vice President, are authorized to receive reimbursement from the Society for travel expenses in connection with their attendance at the annual meetings.

5. AUDITING COMMITTEE

An auditing committee shall be appointed by the President each year which shall, prior to the annual meeting, audit the accounts of the Treasurer and the Business Manager, and certify their audit in a written report to the Society at the final business session. The Council may at its discretion authorize an audit by a certified public accountant as well as an auditing committee. Such audit shall be published in the official journal.

6. SUBMISSION OF PAPERS AND ABSTRACTS

Members who wish to present papers at the annual meeting must submit to the Secretary three copies of each abstract. These abstracts must be clear and concise, contain no tabular data, and not exceed 200 words in length. They should include only statements of fact, unpublished information, and directly derived conclusions or hypotheses. Reports of progress, or of disease occurrences, or of routine tests of ordinary control measures, are not desired, unless new and significant developments are clearly indicated.

(a). *Date due.* Abstracts must be received by the Secretary not later than 60 days prior to the annual meeting. The Secretary shall return to the author abstracts received after the closing date. Members are requested not to submit abstracts unless they expect to attend the meeting.

(b). *Number.* Each member is limited to two papers on which he may appear as sole, senior, or junior author.

(c). *Editing and Reviewing.* Abstracts are to be reviewed by a committee appointed annually by the Editor-in-Chief of PHYTOPATHOLOGY, and this committee is directed to reject or return to authors for revision such abstracts as fail to meet the stated requirements.

(d). *Time Limit on Papers.* Members are requested to limit presentation time to 5 to 10 minutes. The maximum time allowed for other than invitation papers will be 15 minutes. Complicated tables or graphs should not be shown.

(e). *Publication of Abstracts.* Abstracts of papers presented at the annual meeting shall be printed in PHYTOPATHOLOGY at the expense of the Society, provided that not more than two abstracts under the sole or joint authorship of one member, that are presented at meetings of the Society or the Divisions, shall be published at the Society's expense within one calendar year.

7. DIVISIONS

The following provisions shall govern the organization and regulation of Divisions of the Society.

(a). *Name.* Divisions shall use the name of the parent Society with appropriate geographical term; for example, The American Phytopathological Society, Pacific Division.

(b). *Membership.* Divisions shall elect to full membership only members of The American Phytopathological Society, but each Division may elect associate members under such rules as it may adopt.

(c). *Publication.* Summaries of the proceedings of the annual meetings of Divisions shall be published in PHYTOPATHOLOGY at the Society's expense. Abstracts of papers presented at Division meetings also may be published in PHYTOPATHOLOGY subject to the rules governing the preparation and publication of abstracts as stated in Rule 6, and with the specific limit of two on the number of abstracts that any member may have published at the Society's expense within one calendar year.

(d). *Constitution.* The constitution or articles of organization of all Divisions shall contain a provision or provisions ratifying the above rules. The constitution of all Divisions shall contain nothing in conflict with the constitution of The American Phytopathological Society. With the exceptions defined by the above rules, the Divisions shall enjoy complete autonomy.

8. AFFILIATED SOCIETIES

The following provisions shall govern the establishment of affiliated relations with other phytopathological societies.

(a). Upon advice to the Council that affiliation is desired, the Council may invite to affiliation with The American Phytopathological Society any other professional society in the field of plant pathology.

(b). The existence of affiliation of other societies with this Society shall be indicated by listing the names of affiliated societies in each number of PHYTOPATHOLOGY.

(c). The Council may at its discretion authorize the publication of summaries of the proceedings of annual meetings of affiliated societies in PHYTOPATHOLOGY.

(d). The Council may at its discretion authorize the publication for limited periods, subject to annual renewal of the privilege, of research papers of members of affiliated societies who are not members of The American Phytopathological Society.

9. SOCIETY REPRESENTATIVES IN OTHER ORGANIZATIONS

The Council is authorized to select, with the approval of the Society, representatives in other organizations in which the Society is entitled to representation. The following are specifically authorized: two representatives on the Council of the American Association for the Advancement of Science for a two-year term; one representative on the Division of Biology and Agriculture of the National Research Council for a three-year term; one member of the Editorial Board of the American Journal of Botany for a three-year term; and one member of the Council of the Union of American Biological Societies for a three-year term.

10. AMENDMENTS

These rules may be amended by a majority vote of the members voting at any regular meeting of the Society, or by a majority vote of the members voting in a ballot conducted by mail.

A BACTERIAL ROOT AND STEM DISEASE OF GUAYULE

W. A. CAMPBELL

INTRODUCTION

In May, 1944, a root disease of guayule (*Parthenium argentatum* A. Gray), with symptoms differing from those caused by *Phytophthora drechsleri* Tucker,[1] was first observed in an irrigated planting near Bakersfield, California. This disease, then termed resinous stem and root rot[2] on the basis of the characteristic black resinous exudate present on diseased roots and stems, has since been demonstrated to be caused by a bacterium and has been briefly described by Campbell and Presley[3] as a bacterial rot of guayule.

During the summer and fall of 1944 the disease was observed in several irrigated plantings in the San Joaquin Valley, California, but was an important cause of loss only in the field where it was first discovered. Diseased plants with similar symptoms were also observed in 1944 on several irrigated indicator plots in Texas. An outbreak of the disease occurred near Patterson, California, in August, 1945. In the same area severe losses from Phytophthora root rot also developed following midsummer irrigations. In some plantations on heavy soils, the combined loss from bacterial root and stem disease and Phytophthora root rot was 20 per cent of the existing stand. The bacterial disease also caused severe losses in several plantings near Bakersfield following heavy irrigations in July and August, 1945.

Losses from bacterial root and stem disease in several irrigated fields occurred mainly in localized areas, which because of topography or location received the most water. In these fields moisture conditions were favorable for the development and dissemination of the pathogen only in low places or at the beginning or end of the irrigation runs where water accumulated. In other fields on soils of good water-holding capacity, the loss was more uniformly distributed and followed heavy irrigations during hot summer weather.

SYMPTOMS

In common with other root diseases of guayule, wilting is usually the first readily recognized symptom of bacterial root and stem disease. The degree and completeness of wilting are determined by the vegetative condition of the plants when attacked and by weather conditions. The bacterium in-

[1] Braun, A. J. Phytophthora root rot of guayule. (Abstr.) Phytopath. 34: 933. 1944.

[2] Guayule plantation diseases and their control. Mimeograph release. Special Guayule Research Project. 19pp. 1944.

[3] Campbell, W. A., and J. T. Presley. Diseases of cultivated guayule and their control. U. S. Dept. Agr. Circ. 749. 1946.

271

vades the root tissue of succulent plants and, consequently, affected plants wilt suddenly, without recovery, during hot weather. Nonsucculent plants frequently exhibit progressive wilting of the branches when attacked. Root lesions develop more slowly than in succulent plants and cause progressive interference with water uptake, and wilting may take place branch by branch. The plants may recover during the night and retain their normal appearance for several days before they permanently wilt. In cool weather diseased nonsucculent plants often do not wilt. Instead the leaves gradually yellow and dry on the plant.

The bark on the lower part of the main branches of recently wilted plants, or those that have been dead for a short time, usually has a brownish or blackish resinous exudate. The amount and distribution of this vary considerably from one diseased plant to another but its presence is a characteristic symptom of bacterial disease. The resinous exudate follows the extension of the lesions into the branches and is at first yellowish or light brown but quickly turns dark brown and eventually black. In the early stage of stem lesions the resin exudate is present as small droplets but these gradually increase in size and amount until the bark is often covered with an extensive resinous coating (Fig. 1, A).

When a diseased plant is pulled from the ground, the root crown and upper tap root usually are covered with an adhering layer of resin and earth (Fig. 1, B). The root lesions differ considerably from those caused by *Phytophthora drechsleri* inasmuch as the diseased tissue beneath the lesion does not become blackened or sunken. Except in the advanced stages following invasion by secondary organisms, the diseased root cortex underneath the resin-covered bark is usually but slightly discolored. Diseased cortex is, however, softer than healthy root tissue and somewhat cheesy in texture. After a few seconds' exposure to the air, the cut surface turns a dirty red or pink before finally becoming brown, in sharp contrast to the olive-green oxidation color imparted to healthy root tissue under the same conditions. Older diseased tissue usually is a dull red-brown but may be almost black in the final stages of disintegration. The pink pigment of the diseased root tissue in its early stages is water soluble and quickly diffuses out when such tissue is placed in water. A pronounced pink or red discoloration is usually present in the cambium and newly formed wood for some distance in advance of the lesions.

On two- and three-year-old plants that are not abundantly supplied with water throughout the summer but are irrigated heavily but once or twice during the season, root lesions may develop to a limited extent. These may be checked by drying but again become active on subsequent irrigations. Therefore losses developing in an area are usually only a fraction of the eventual loss since further mortality generally takes place following irrigations of moderate amounts. In all observed instances of severe loss from bacterial disease the original outbreak followed heavy irrigations.

ORGANISMS ASSOCIATED WITH BACTERIAL DISEASE

During the two growing seasons that the disease has been under observation numerous isolations have been made from root and stem lesions.[4]   A
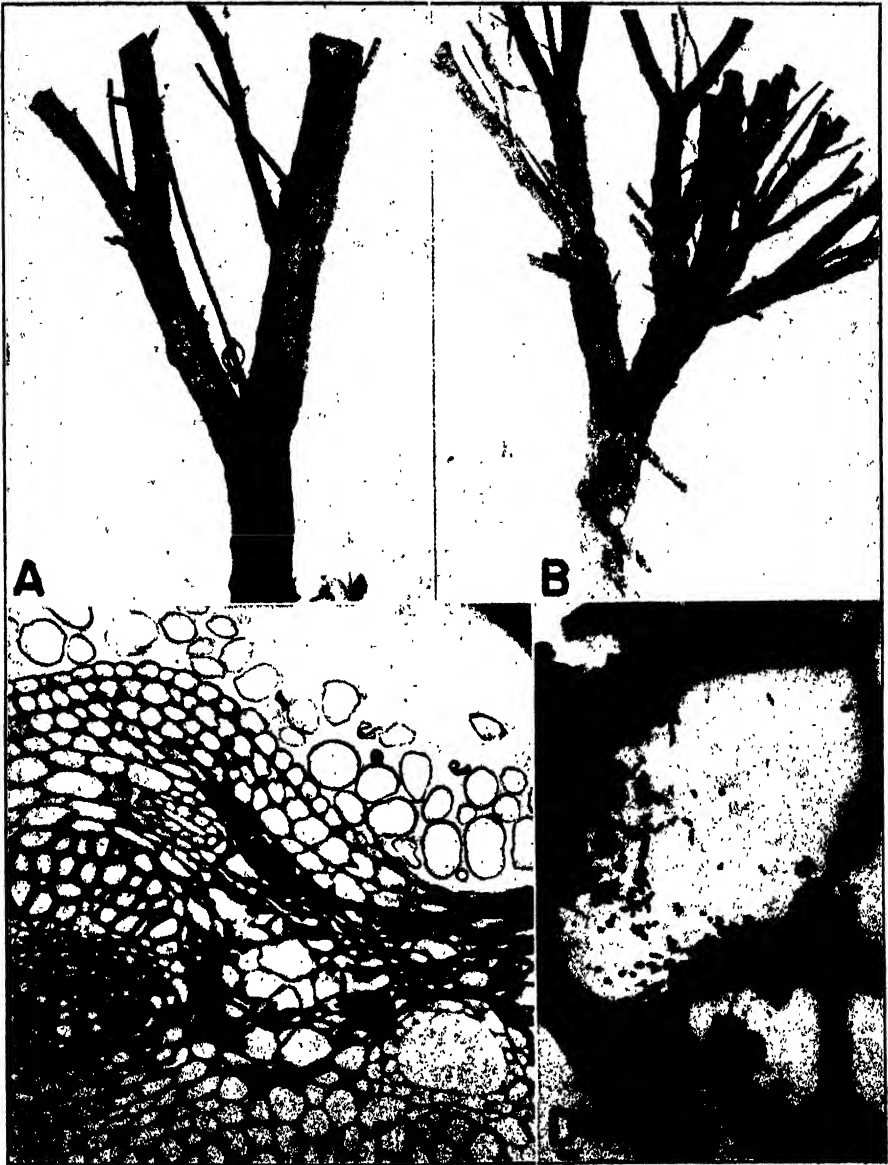
FIG. 1. Bacterial root and stem disease of guayule caused by *Erwinia sp.* A. Resinous exudate on stem. Approximately natural size. B. Resinous exudate on root crown, tap root, and stems of 3-year-old diseased plant. Approximately ½ natural size. C. Cross-section of diseased stem showing breakdown of cortical cells. D. Bacteria in diseased cells.

[4] Many of the isolations were made by Lois Weston Weeth and A. J. Braun.

variety of fungi were commonly obtained from lesions at the root crown or below.   These include two species of *Fusarium*, *Alternaria sp.*, and several representatives of the *Mucorales*.   None of these fungi were pathogenic to guayule in inoculation experiments in the greenhouse.

Bacteria were also present in the root lesions but were usually obscured by rapidly growing fungi.   Isolations from the uppermost lesions in the stems invariably gave pure cultures of the bacterium.   The presence of this organism in diseased cortical tissues could also be demonstrated by microscopic examination.   The guayule bacterium has been identified by Starr[5] as an *Erwinia sp.*

INOCULATION EXPERIMENTS
Proof of Pathogenicity

Repeated inoculation experiments have demonstrated the pathogenicity of the *Erwinia sp.*, isolated from guayule, to guayule seedlings in the greenhouse at temperatures ranging from 70° to 90° F.   In a representative experiment eight 6-month-old transplants, 4 plants to a can, in two 5-gallon cans of pasteurized soil were inoculated on October 26, 1945, with a pure culture of the bacterium grown on potato-dextrose agar.   Inoculation was by means of a longitudinal cut, one-half inch long, through the bark at and just below ground line.   A small amount of bacteria and agar was placed in the cut, after which cotton was bound over the area and the soil replaced.   The checks were treated similarly with sterile potato-dextrose agar.   Following inoculation the plants were saturated for 24 hr. and watered 2 or 3 times a day thereafter.   Five out of 8 inoculated plants were killed by November 16, and the remaining three were pulled on that date for examination.   Two of these had large lesions on the roots but the leaves had not wilted; the third had a small root lesion that had not progressed into the stem.   The bacterium was recovered in pure culture from diseased tissue in the stems.   No other organism was present.   The four check plants remained healthy and when the roots were examined approximately 3 weeks after inoculation with plain agar, the wounds were found to be completely healed.

Root Injuries and Infection

In the foregoing experiment the bacterium was introduced into the root tissue through a cut in the bark and in massive amounts.   In another experiment to determine whether or not the bacterium could penetrate uninjured bark, 8 plants were inoculated by applying bacteria on potato-dextrose agar directly to uninjured bark just below the root crown.   Four check plants were similarly treated with plain agar.   Cotton was placed over the inoculations and the soil was pressed over the cotton.   The soil in the cans was kept continuously soaked for 24 hr. following inoculation and watered twice a day thereafter.

After two months 3 of the inoculated plants had definite root lesions,

which extended into the stems. Five plants remained healthy as did the 4 check plants inoculated with plain agar.

In further experiments on the relation of wounds to infection, 6-month-old plants growing 4 to a 5-gallon can were used. In each can 2 of the 4 plants were injured just below ground line by several small cuts made in the bark by a sharp scalpel and 2 were left uninjured. Inoculation was by means of a suspension of bacteria in distilled water, followed by heavy watering for 24 hr. and twice a day thereafter. The check plants were flooded with distilled water immediately after wounding.

The four plants injured at the ground line and flooded with a suspension of bacteria in water wilted in from 1 to 2 weeks after inoculation. The four noninjured plants remained healthy as did the injured and noninjured check plants. The wounds on the roots of the injured checks had healed in 3 weeks at which time all of the plants were pulled for examination.

In a repetition of the same experiment with 8 succulent and 8 hardened plants, 15 out of 16 plants in both categories with injuries at the crown and flooded with bacteria succumbed to the disease and 1 remained uninfected at the end of 2 weeks. Two noninjured hardened plants out of 8 inoculated became infected, and all 8 inoculated noninjured succulent plants remained healthy. There appeared to be little difference in the susceptibility of injured succulent and hardened plants under the conditions of the experiment. However, the succulent plants wilted more quickly than the hardened plants when attacked.

Temperature and Infection

In order to determine the effect of temperature upon the pathogenicity of *Erwinia sp.* from guayule, inoculations were made on 7-month-old guayule seedlings at 9 constant temperatures ranging from 55° to 98° F. All of the plants used were of variety 593 and were in approximately the same degree of succulence when placed in the temperature tanks. Six plants were maintained at each temperature. These were inoculated on January 11 by applying a half-inch square portion of isolate 263C on nutrient-dextrose agar directly to small cuts through the bark 1 inch below the soil surface. After inoculation the plants were watered at frequent intervals in order to keep the soil continuously moist. The water was heated or cooled to the proper temperature before applying. The results of the experiment are summarized in table 1.

The bacterium from guayule readily caused infection of guayule at temperatures from 70° to 98° F. Two plants out of 6 were infected at 64°, indicating limited ability to cause infection at this temperature during a period of 14 days. At both 55° and 60° the inoculation wounds were either healed or were healing. Judging from the rapidity with which external disease expression developed as exemplified by wilting, the guayule bacterium was most virulent from 75° to 85° inclusive. The slowness of disease expression at 70° was due to the lower transpiration rate at that

temperature and consequently the leaves did not wilt until the cortex was badly decayed. At 89° and 98° the plants had become somewhat hardened and consequently wilting was slower and less evident than at the temperatures at which the plants were growing more vigorously. In addition, at these high temperatures the disease was confined to the root and the bacteria did not invade the stem except for a short distance above the ground. At lower temperatures the cortex of the branches was softened even to the ends of the branches before permanent wilting occurred.

Check plants grown at the same temperatures and inoculated with nutrient-dextrose agar remained healthy during the 14 days. In most plants the inoculation wounds had completely healed.

Tissue Affected

In order to determine the stem tissues invaded by the bacterium sections for microscopic examination were taken at various heights on the stems of diseased plants. These were fixed in formalin–propionic acid–ethanol solu-

TABLE 1.—*Effect of constant temperatures upon the time required for infection of guayule by Erwinia sp. isolated from guayule*

Soil temperature[a]	Total plants inoculated	Plants diseased in 14 days	Time required for first disease expression[b]	Average time for complete wilting per plant
° F.	Number	Number	Days	Days
55	6	0	0	....
60	6	0	0	. ...
64	6	2	7	....
70	6	6	8	13
75	6	6	5	7
80	6	6	4	7
85	6	6	6	8
89	6	6	9	12
98	6	6	11	. . .

[a] At one inch depth, range plus or minus 1° F.
[b] As indicated by slight wilting of leaves.

tion, embedded in paraffin, sectioned, and stained by the usual histological methods.[c] Because root tissues quickly became badly disorganized following infection, the histological studies were confined to the stems.

The bacterium invades primarily the cortex, causing a breakdown of the cortical parenchyma (Fig. 1, C). The resin flow commonly observed on diseased stems resulted from the breakdown of the cells around the resin canals and consequent release of their contents, which escaped to the outside through the disintegrated cortical tissue. The bacterium could be readily demonstrated in large numbers in the diseased cortex (Fig. 1, D). The upward progress of the bacteria through the cortex took place through intercellular spaces and by passage from one diseased cell to another.

[c] The author is greatly indebted to L. C. Erickson for the histological sections and photomicrographs.

SUMMARY

A bacterial root and stem disease of guayule caused by an *Erwinia sp.* caused considerable loss in irrigated plantations in the San Joaquin Valley of California in 1944 and 1945. Losses from bacterial disease were generally associated with heavy summer irrigations. In several fields losses were restricted to low places where water accumulated but in others the losses were more uniformly distributed over the area.

In the field, infections developed at or below the root crown, presumably through injuries of various kinds. Root and stem lesions were frequently but not always accompanied by copious resinous exudation. Symptom expression was influenced by temperature and type of growth. Succulent plants wilted suddenly but hardened plants frequently wilted branch by branch or the leaves dried on the plant without pronounced wilting.

The pathogenicity of the organism was demonstrated by inoculation experiments in the greenhouse. The average time required for complete wilting of inoculated plants at constant temperatures of 75°, 80°, and 85° F. was 7, 7, and 8 days, respectively. Disease expression was slower at 70°, 89°, and 98° F. Below 60° F. the inoculation wounds healed without infection.

Histological studies of diseased stems demonstrated that the bacteria were confined to the cortical cells. These quickly disintegrated in the presence of the bacteria and permitted the bark to separate readily from the stems.

SPECIAL GUAYULE RESEARCH PROJECT,
    SALINAS, CALIFORNIA.

DODDER AS AN AID IN TESTING SOME PLANT SPECIES FOR CURLY-TOP VIRUS

N. J. GIDDINGS[1]

Some plant species highly susceptible to curly top are very unsatisfactory hosts for the beet leafhopper (*Eutettix tenellus* Baker). Several species of *Nicotiana* and the commercial varieties of tomato are in such a grouping. The leafhoppers get their legs and wings entangled with the hairs or by the sticky exudate and may live only two or three days under warm, favorable, feeding conditions. During the time that they are caged on such plants they are not likely to feed well.

It has been found that one or more species of dodder thrive well upon such plants and that the leafhoppers feed well upon dodder. The leafhoppers also survive very well in cages which include a reasonably strong growth of dodder and not many leaves of the plant that is an undesirable host for them. The dodder takes up curly-top virus along with nutrients from its host, as reported by Bennett,[2] and the leafhoppers readily pick up the virus from the dodder. The method is illustrated by the pictures in figure 1.

During 1938–39 several groups of previously inoculated Turkish tobacco plants were tested for curly-top virus using young sugar beets as test plants and one leafhopper per plant for inoculation. Five groups were tested without the aid of dodder and three groups with *Cuscuta subinclusa* Dur. and Hilg. The results are given in table 1.

TABLE 1.—*Results of tests for curly-top virus in inoculated plants of Nicotiana tabacum L.*

	Without dodder				With dodder		
Source plants	Test plants			Source plants	Test plants		
	Inoculated	Diseased			Inoculated	Diseased	
No.	No.	No.	Pct.	No.	No.	No.	Pct.
8	59	5	8	4	60	30	50
7	44	4	9				
16	189	35	19	11	126	65	52
9	85	25	29				
9	108	29	27	9	108	59	55
Tot. 49	485	98		24	294	154	
Av. Pct.			20				52

[2] Bennett, C. W. Dodder transmission of plant viruses. Phytopath. 34: 905–932. 1944.

FIG. 1.   The use of dodder on *Nicotiana tabacum* L.   A. Strand of dodder in bottle of water just getting established upon tobacco.   B. Dodder growing vigorously on tobacco. C. Tip and most of adjacent leaves cut off so that leafhoppers will have less interference from them while feeding on dodder.   Cotton will be wrapped around tobacco stem just above string.   D. Celluloid cage tied on over dodder.

Of the 49 plants tested without dodder, 45 per cent failed to give infection in any of the test plants, while among the 24 plants tested with dodder, 25 per cent failed to give infection in any of the test plants. There was more than two and one-half times as much infection among the test plants when dodder was used on the tobacco than when the test was made using leafhoppers which had to feed directly on the tobacco.

During 1939 a comparative test was made using inoculated plants of *Nicotiana glutinosa* L. In this experiment, the same plants were first tested without the dodder and then with *Cuscuta subinclusa*. The results are given in table 2.

TABLE 2.—*Results of tests for curly-top virus in inoculated plants of Nicotiana glutinosa L., 1939*

Source plant number	Without dodder			With dodder		
	Test plants			Test plants		
	Inoculated[a]	Diseased		Inoculated[b]	Diseased	
	No.	*No.*	*Pct.*	*No.*	*No.*	*Pct.*
82	15	1	7	12	5	42
83	21	1	5	12	10	83
84	15	0	0	12	0	0
85	25	2	8	12	5	42
86	23	1	4	12	8	67
87	22	0	0	4	1	25
88	20	10	50	11	11	100
89	32	0	0	12	10	83
90	22	2	9	12	10	83
91	20	2	50	12	11	92
92	24	5	21	12	11	92
93	19	6	32	12	12	100
95	12	0	0	10	4	40
113	12	8	67	11	9	82
Totals	282	38	...	156	108	...
Av. Pct.			13			69

[a] Test plants were inoculated July 10, 1939.
[b] Test plants were inoculated August 11, 1939.

Three of the *Nicotiana glutinosa* plants gave negative tests without the dodder, but positive tests when dodder was used. There was more than five times as much infection among sugar beet test plants when dodder was used than when it was not used.

The method has been used many times since the above comparisons were made, and it has been very satisfactory if care was exercised to see that the dodder was well established and growing vigorously upon the host plant to be tested.

U. S. DEPARTMENT OF AGRICULTURE,
   RIVERSIDE, CALIFORNIA.

THE CAUSAL AGENT OF BACTERIAL ROOT AND STEM DISEASE OF GUAYULE

MORTIMER P. STARR[1,2]

Late in 1945, the writer was consulted about the identity of the bacterium which Campbell (5) had shown to cause a root and stem disease of the rubber-bearing plant, guayule (*Parthenium argentatum* A. Gray). Cultures of bacteria isolated from naturally and artifically infected guayule were received from W. A. Campbell in December, 1945. It is the purpose of this communication to describe these cultures and to discuss the taxonomy and nomenclature of this organism.

METHODS

The purity of all cultures was assured by microscopic examination, and by plating from dilute aqueous suspensions onto yeast agar. From isolated colonies, the cultures were restreaked on yeast agar, and from colonies derived from these second platings, a third streaking was prepared in the same way. All of the second and third plates showed but one type of colony. A single colony was fished from each plate of the third series and shown to be capable of inducing typical symptoms (5) when inoculated into guayule plants. These pure and virulent cultures (referred to hereafter as "original pure cultures") were then run through the determinative tests which are presented below.

In general the methods employed are those given in the Manual of Methods for Pure Culture Study of Bacteria (15), and in the Manual of Dehydrated Culture Media and Reagents (6). When a modification, or a technique not described in either of these publications, was used, appropriate citation or description of the method is given.

Ten isolates and reisolates, and sometimes a larger number, were used in all tests; unless otherwise indicated, all cultures behaved similarly. Except where stated otherwise, cultures were incubated at 28° C.

DESCRIPTION OF THE PATHOGEN

Morphology and Staining Reactions

The guayule pathogen is rod-shaped. From 18-hour-old yeast-agar or broth cultures, the cells are cylindrical with hemispherical ends, and about 0.7–0.8 by 3–7 μ or sometimes longer. Older cells are shorter and thinner and include, at times, curved, club-shaped, spindle-shaped, and other involution forms. The cells usually occur singly, and sometimes in pairs.

Young cells stain well with gentian violet, fuchsin, and methylene blue. Older cells stain poorly even after long exposure to gentian violet and fuchsin; methylene blue is a somewhat better stain for such old cells. The guayule pathogen is Gram-negative by both the Hucker and the Kopeloff and Beerman modifications of Gram's stain. This statement is based upon examination of cultures, from both sugar-free and sugar-containing liquid and solid media, ranging in age from 12 hours to 3 months. The guayule pathogen is not acid-fast.

The guayule pathogen is actively motile, motility being retained for more than a week in many cultures. By means of the Zettnow (11) and the Gray flagella stains, peritrichous flagellar arrangement can be demonstrated; usually there are 6 to 12 flagella per cell, each about 1.5 to 2 times as long as the bacterium.

Endospores are not formed. Although some of the more lightly staining older cultures contain cells which are superficially spore-like in that the center of the cell remains unstained while the poles and periphery stain, these cells cannot be stained by Dorner's or Ziehl-Neelsen's spore stains, the central regions are not refractile like spores, nor do they survive heating to 85° C. for ten minutes as do true endospores. Inasmuch as stained preparations from young cultures sometimes show polar areas more deeply colored than the centers, the "spore-like" cells may come about as a result of the over-all reduced stainability of the older cells. These central areas do not stain with iodine.

Capsules are seen about the cells in many preparations, notably in smears stained by Gray's flagella stain. By means of Hiss's capsule stain, narrow capsules could be demonstrated surrounding cells from 7-day-old dextrose-yeast-agar cultures.

Cultural Characteristics

Colonies on yeast agar and on beef-peptone agar.—The yeast agar was made with 1 per cent Bacto yeast extract and 2 per cent Bacto agar, and its pH was 6.8. The beef-peptone agar contained 0.3 per cent Bacto beef extract, 0.5 per cent Bacto peptone, and 2 per cent agar; its pH was 6.8. Surface colonies are flat, greyish, iridescent, dry and not glistening, almost transparent, approximately round, with amoeboid edges. The surfaces of most colonies appear wrinkled to the naked eye; at magnifications of 50–100 ×, shallow, irregular furrows can be seen, and the centers and peripheries appear a little raised. The texture is amorphous or finely granular

at 150 ×. The growth is readily suspended in water and is not viscid. Well-isolated surface colonies are ordinarily under 1 cm. in diameter. Subsurface colonies are lenticular, opaque, and usually about 1 mm. long.

Colonies on yeast-dextrose agar (yeast agar plus 2 per cent dextrose).— Surface colonies are slightly raised, with light tan or cream centers and practically colorless peripheries, round with amoeboid edges, translucent, not mucoid or viscid. The surface is wrinkled or stippled, and the size usually under 1 cm.

*Colonies on yeast-dextrose-CaCO₃ agar—"YDC" (yeast-dextrose agar plus 2 per cent precipitated chalk).—*Colonies on this medium are generally similar to those on yeast-dextrose agar. The CaCO₃ is decomposed, more so at times than others, resulting in a cleared space beneath the colony not ordinarily extending beyond the edge. At times, a layer of redeposited CaCO₃ can be seen at the margin of the colony.

By plating the original pure cultures on this medium, there could be obtained from each culture two colony types which differ in the rate and extent of clearing of the CaCO₃. The more extensively decomposing sort (type A) is very mucoid on this medium, although the slime seems to dry out after a few days. In addition, colonies of type A appear somewhat tan by contrast with the less extensively decomposing type B, because of the complete dissolution of the chalk beneath the type A colony with the result that the brown color of the medium is visible through the translucent growth. Types A and B differ in other characteristics, notably in reaction on eosin-methylene blue agar, in milk, and in broth, as described in the appropriate places below. This phenomenon probably represents a transition somewhere in the series Rough→Smooth→Mucoid, with a concurrent change in the mode or rate of metabolism of sugar.

Unless otherwise specified, the descriptive matter presented herein refers to the original pure cultures, which were probably entirely in the type B condition.

*Eosin–methylene blue agar—"EMB."—*Two types of reaction are observed on this Bacto medium containing both lactose and sucrose: the more common is formation of colonies similar in size and shape to those seen on yeast-dextrose agar, although sometimes more moist and spreading; the color is pink, often with blue or violet centers, sometimes becoming entirely blue or violet. The other reaction is production of dark violet colonies which have a green-metallic sheen by reflected light, and are often exceedingly mucoid. Some of the original pure cultures yielded a large proportion of greening colonies; in others they were rare or absent. From papillae on nongreening colonies and on yeast-agar-slant growth, or by repeated plating of each of the original pure cultures on EMB, there could be isolated more or less pure strains of the greening variety. Both the greening and the nongreening sorts were unstable, and reverted to mixtures; the nongreening type seemed the more stable of the two. At the same time, the two colony types on YDC, discussed in the paragraphs above, had been

found. Cultivation of the EMB varieties on YDC, and of the YDC types on EMB, showed that they are probably duplicates; the greening variety corresponding to type A, and the nongreening sort to type B. Both EMB varieties proved to be virulent to guayule and neither of them fermented filter-sterilized lactose any more quickly than did the original pure cultures. However, type A did produce an acid reaction in autoclaved litmus milk, in contrast to the alkaline peptonization brought about by the type B cultures.

Growth on yeast-agar slants.—Flat, practically colorless, not spreading, with irregular margins, iridescent, not viscid. At times, and especially on moister slants, the growth becomes heavier, whiter, more spreading and wet-looking. This may be related to the changes noted for colonies on YDC.

Growth on yeast-dextrose-agar slants.—Wet-looking, whitish, slightly raised, abundant, spreading, iridescent, not mucoid or viscid.

Growth on yeast-dextrose-$CaCO_3$-agar slants.—Flat, colorless to cream, with slightly irregular edges, dry and not glistening. This is probably the type B growth; nothing like type A was seen on these slant cultures, but no special effort was made to secure this modification.

Yeast or beef-peptone broth.—Definitely turbid within 12 hours. On further incubation of type B cultures, the turbidity increases, a finely-granular, very thin, easily dislodged pellicle forms, and a smooth or finely granular sediment is deposited. Type A cultures have heavier sediments and are less turbid than type B.

Yeast-dextrose broth.—Heavy turbidity, abundant viscid or slightly granular sediment, white ring, easily dislodged granular pellicle. The type A cultures have generally a more abundant and more granular sediment and are less turbid than type B. In the better-buffered dextrose-peptone broth used for the Voges-Proskauer tests, growth is somewhat heavier and these differences between types A and B are accentuated.

Action on autoclaved litmus milk (Bacto), on autoclaved brom cresol purple milk, and on autoclaved skim milk.—Peptonization starts in the type B cultures after about a week and continues until the entire tube is cleared—meanwhile, the indicator shows an alkaline reaction. The type A cultures usually make an acid curd after 10 to 14 days, the litmus is reduced, and no peptonization occurs.

Potato cylinders.—No growth, or only a trace of growth, on the surface of the potato. Moderate growth in the liquid below the potato.

Relationship to oxygen.—In thioglycollate broth (Bacto) and in yeast-agar shake cultures, heavy growth occurs only in the upper 1 to 2 mm., with no growth below, showing a distinct preference for aerobic conditions. In yeast-dextrose-agar shake cultures, there is growth throughout the tube, and the agar is blown apart by gas formation, illustrating the organism's ability of growing anaerobically by means of its fermentative metabolism. The latter capacity is revealed also by growth and the formation of acid and gas in a synthetic dextrose medium (17), within the closed arm of Smith fermentation tubes.

Catalase.—Catalase in cultures on various media can be shown by the evolution of gas when H_2O_2 is added.

Chromogenesis.—Other than occasional light tan or cream growth on some agar media, there has been observed no distinct coloration of the cell mass or medium.

Carbon and Nitrogen Metabolism

Minimal nutritive requirements.—The guayule pathogen grows moderately well from small inocula in a synthetic medium (17) containing NH_4Cl, $MgSO_4$, phosphate buffer, "trace" elements, and separately sterilized, purified dextrose. More rapid growth and a somewhat larger crop results from the addition of 0.5 per cent "vitamin-free" casein hydrolyzate. At least part of this apparent stimulation is due to the buffering effect of the amino acids or their degradation products which maintain the pH at a level suitable for continued growth; the limiting pH of 4.2 was reached in a few days in the NH_4Cl-dextrose medium, whereas, even after 20 days' incubation, the pH in the casein hydrolyzate medium was only about 5.0. A mixture of known vitamins has no detectable effect upon growth.

Utilization of sodium citrate as sole carbon source.—All cultures grow moderately well on Bacto Simmons citrate agar and the pH-indicator shows an alkaline reaction, thus indicating that the guayule bacterium can use citrate as sole carbon source.

Action on sugars.—The ability to dissimilate sugars was determined in a medium containing 0.3 per cent beef extract, 0.5 per cent peptone, Andrade's indicator; pH 6.7. Sugars were sterilized by passage through Seitz filters and were used in final concentrations of 1 per cent in Durham tubes. Acid and gas (generally under 10 per cent) are formed within 48 hours from arabinose, dextrose, galactose, levulose, maltose, raffinose, salicin, sucrose. Somewhat slower production of acid and gas takes place from cellobiose (7 days), lactose (14 to 20 days), rhamnose (7 days), xylose (2 to 4 days). Acid, but no detectable gas, is formed slowly from inositol. No acid or gas is produced after a month's incubation from dextrin, inulin, mannitol, sorbitol, trehalose, and sugar-free base. In many cases, an originally acid reaction became alkaline after longer incubation.

Methyl red and Voges-Proskauer tests (Bacto M.R.–V.P. medium).—After 48 hours there is a negative methyl red test, which remains negative for a month. The Voges-Proskauer test is weakly positive or questionable for the first week, later becoming more distinctly positive. Acetyl-methylcarbinol was sought by adding a few drops of $FeCl_3$ solution and 5 ml. of 10 per cent KOH to 5 ml. of culture; the tests were incubated at 37° C. for several hours before the final readings were made.

Action on starch.—The starch agar had 0.5 per cent yeast extract, 0.2 per cent soluble starch, and 2 per cent agar; its pH was 6.8. Starch broth was the same, minus agar. Good growth on the starch agar, but no action on the starch was detectable by flooding the plates with Lugol's iodine solution after 3, 7, or 20 days. Lack of diastatic power was verified in the liquid

medium, in which there is good growth, but a negative iodine test, and no acid or reducing sugar, after 2, 7, or 12 days. .

Action on sodium ammonium pectate.—A pectate medium was prepared by dissolving 3 g. of sodium ammonium pectate (No. 24 of California Fruit Growers Exchange, Ontario, California) in 100 ml. distilled water to which had previously been added 0.9 ml. normal NaOH, 0.6 ml. 10 per cent $CaCl_2 \cdot 2H_2O$, and brom thymol blue as indicator. After complete solution on a water bath, the pH was adjusted to 7.3, the medium tubed and autoclaved, after which the reaction was pH 6.4. A yeast-pectate medium was prepared in the same way by adding 1 per cent yeast extract to the above. After the media had gelled, they were inoculated by stabbing. The guayule pathogen grows well within 24 hours in both media, and with particular luxuriance in yeast-pectate. After 2 days, the growth occupies a carrot-shaped space reaching to the bottom of the tube, and both media are liquefied, ranging, with the different isolates, from a few millimeters at the surface to practically complete liquefaction. After a week or 10 days, all cultures are completely liquid and the reaction, which at first is acid, becomes neutral.

Pectolytic action on slices of raw carrot.—All cultures produce an active, soft rot of raw carrot slices within 2 days.

Action on guayule resin.—On Allen, Naghski, and Hoover's (1) resin agar and on resin-yeast agar there is good growth, but no decomposition of the guayule resin on either of these media after 7 days.[3] Resin-decomposing bacteria tested at the same time brought about distinct clearing of the resin about the colonies.

Action on gelatin.—The medium contained 0.3 per cent beef extract, 0.5 per cent peptone, and 12 per cent Bacto gelatin; pH was 6.6. A second batch contained 1 per cent yeast extract, 12 per cent Bacto gelatin; pH was 6.0. In both media, there is a funnel-shaped liquid zone at the end of 2 days at 22° C,; within a week or 10 days, the entire tube is liquefied.

Indole production.—On 1 per cent Bacto tryptone at pH 7.0 indole is formed in 2 days as shown with Kovács' reagent (2).

Hydrogen sulphide production.—Questionable in Bacto lead acetate agar. Slight browning, appearing in 3 days but not progressing much thereafter, of lead acetate paper suspended over cultures in 1 per cent tryptone, or on yeast-agar slants.

Reduction of nitrates.—On a medium of 0.3 per cent beef extract, 0.5 per cent peptone, 0.1 per cent KNO_3, 1.2 per cent agar, at pH 6.8, a strongly positive test for nitrites is obtained after 2 days using the sulfanilic acid and α-naphthylamine reagents.

Tyrosinase production.—Tyrosine and control media (16) were modified by omitting the agar and adding 0.1 per cent glutamic acid; pH was 7.8. Abundant growth, but no definite coloration of either medium after 30 days, indicating the absence of tyrosinase.

ATTEMPTS TO INFECT GUAYULE WITH OTHER SOFT-ROT BACTERIA

After the determinative tests made it appear likely that the guayule pathogen was a species of *Erwinia,* several attempts were made to infect guayule plants with virulent soft-rot bacteria of that genus isolated from other hosts. The following eleven isolates[4] were studied: five isolates of *Erwinia carotovora,* one isolated from cabbage and sent by E. H. Garrard, the others isolated from yellow calla, carrot, celery, and larkspur and received from P. A. Ark; two isolates of *E. aroideae,* one sent by E. H. Garrard, and the other isolated from tobacco stems and received from W. J. Dowson; *E. oleracerae,* isolated from cauliflower and sent by E. H. Garrard; *E. rhapontici,* isolated from rhubarb and sent by W. J. Dowson; *E. solanisapra* and *E. atroseptica,* both isolated from potato and sent by E. H. Garrard.

In conducting these tests, greenhouse-grown guayule seedlings were used; these had been cultivated in sterilized soil in 6-inch pots. Each isolate was inoculated into 4 to 9 plants. Inoculations were made approximately an inch below the soil surface in the following manner: The soil was removed from the root crown and upper tap root, and a series of small punctures made in the bark with a sharp-pointed scalpel. Bacteria, grown on nutrient-dextrose agar, were applied to the wounds, the soil replaced, and the plants watered immediately. The checks were treated with sterile agar. At least one virulent strain of the guayule pathogen was included in each series as a positive control. After inoculating, the plants were watered every 2 or 3 hours for the first two days, and then 3 times a day thereafter.

In the first series of tests, with Ark's isolates of *Erwinia carotovora,* inoculated plants were kept in tanks at nine different soil temperatures between 13° C. and 37° C., in an effort to provide optimal conditions for infection by these organisms (5). In later tests, with the other isolates of *Erwinia,* plants were held at the usual greenhouse temperatures, since these temperatures had, by that time, been shown to be well within the range for consistently successful infection of guayule by the guayule pathogen.

None of the soft-rot bacteria from hosts other than guayule, when inoculated into guayule, was able to cause the disease under consideration.[5] The check plants, to which only sterile agar had been applied, remained uniformly healthy. The positive controls, inoculated with the guayule pathogen, quickly came down with the typical disease (See figure 1). Since the soft-rot cultures that were used are fairly representative of the entire group, it is probably correct to consider the guayule bacterium unique in the ability of causing the root and stem disease of guayule.

---

[4] The cultures are listed by the names under which they were received. The writer wishes to express his thanks to the donors of these cultures.

[5] Dr. W. A. Campbell, who very kindly conducted and interpreted these tests, reported that a total of three plants, out of the eight that had been inoculated with *Erwinia atroseptica* and Dowson's *E. aroideae,* "had a soft-rot of the root, which could be caused by drowning (13), but none had typical symptoms of guayule rot; *i.e.,* resin flow on lower stem and progress of rot into stem."

TAXONOMY AND NOMENCLATURE

The question of assigning a name to the guayule pathogen is a perplexing one. In accordance with current American usage, as in Bergey's Manual of Determinative Bacteriology, this organism undoubtedly belongs, with other peritrichous, nonsporulating, pectolytic bacteria, in the soft-rot section of the genus *Erwinia*. By Dowson's (7) classification, which has merit, it would be considered a species of *Bacterium*. In other, less generally accepted, and, by certain standards, unjustified, systems, it would be placed in *Paracolobactrum* (4) or *Pectobacterium* (20).

While disposition as to genus is relatively easy, considerably more difficulty attends allocation of this organism to a species. Opinion on the

Fig. 1. Guayule plants in constant temperature tanks at 27° C. (right half) and 30° C. (left half). The plants in the first (bottom) row were inoculated with strain 263C of the guayule pathogen and had typical advanced symptoms of the root and stem disease; the controls (second row) were wounded but treated only with sterile agar; the plants in the third and fourth (top) rows were inoculated with the cultures of *Erwinia carotovora* originally isolated from carrot, yellow calla, larkspur, and celery by P. A. Ark. The control plants and those inoculated with the *E. carotovora* strains from hosts other than guayule were healthy and the inoculation wounds had healed. Inoculated Jan. 31, 1946, and photographed by W. A. Campbell Feb. 21, 1946.

speciation of soft-rot bacteria ranges all the way from realistic arrangements wherein all strains of the soft-rot *Erwinia* are lumped into one or two flexible, but variously described, species, to schemes in which a large number of so-called species are separated on the bases of more-or-less justifiable pathological, serological, biochemical, and cultural distinctions. (See references 3, 7, 8, 10, and 20, for discussions of this subject.)

Naming a bacterium of plant disease origin often brings to the fore the conflict between the "utilitarian" and the "scientific" aspects of microbial taxonomy (19). The practicing plant pathologist is primarily concerned

with the disease-producing abilities of the bacterium and, in so far as possible, prefers nomenclature which in some way indicates this pathogenicity. The fulfillment of this perfectly reasonable, but what must be recognized as "utilitarian" aim has often led to considerable synonymy when the microorganisms are considered bacteriologically apart from their pathological properties. Much confusion and polemic would be avoided if both needs were satisfied—that is, if the nomenclature reflected "scientific" bacteriological relationships as well as "utilitarian" phytopathogenic abilities. The guayule pathogen is a case in point: as a bacteriological unit, it differs not at all from the range of strains which constitute *Erwinia carotovora*; nor, for that matter, is it unlike the group of saprophytic slow-lactose-fermenting "aberrant coliforms" (18). Granted, the guayule pathogen does have cultural properties which, according to various published descriptions, might differentiate it from the average strain of *E. carotovora* (for example, indol production, peptonization of milk, failure to grow on autoclaved potato); however, these distinctions, which in some quarters would be used to separate "species," are well within the limits of variability of the coliform species.

On the other hand, considered from the pathologist's viewpoint, the guayule bacterium does attack guayule, whereas that plant is not infected by virulent soft-rot isolates from other hosts. Although it is debatable whether such host specificity is sufficient justification for erecting a bacterial species, this characteristic is of prime practical importance to the plant pathologist. Incorporation of the guayule pathogen into one of the present *Erwinia* species, without in some way indicating its special ability of infecting guayule, would, therefore, be unwise.

The dilemma can be met by naming the guayule pathogen as a special form or pathogenic variety of *Erwinia carotovora*. There is ample precedent for such action (9, 12, 14). The term "variety" has had two uses in the classification of phytopathogenic bacteria; firstly, to distinguish culturally different organisms which cause indistinguishable diseases on the same host, and secondly, to differentiate organisms which are considered culturally identical, but capable of infecting different hosts. It is suggested that "variety" be restricted to labeling cultural differences, and that the term *"forma specialis"* be used, in the sense recommended in the International Rules of Botanical Nomenclature (See 9), for distinguishing "within the species special forms characterized by their adaptation to different hosts."

In accordance with the foregoing discussion, the name *Erwinia carotovora* f. sp. *parthenii*, f. sp. nov., is suggested for the above-described guayule pathogen. (Synonym: *Bacterium carotovorum* f. sp. *parthenii*.)

SUMMARY

The bacterium which causes a root and stem disease of the rubber-bearing plant, guayule (*Parthenium argentatum*), is described and its sys-

tematic position is discussed.   The name *Erwinia carotovora* f. sp. *parthenii*, f. sp. nov., is suggested for this organism.

BIOLOGY DEPARTMENT,
    BROOKLYN COLLEGE,
        BROOKLYN, NEW YORK.

LITERATURE CITED

1. ALLEN, PAUL J., J. NAGHSKI, and SAM R. HOOVER. Decomposition of guayule resins by microorganisms. Jour. Bact. 47: 559–570. 1944.
2. American Public Health Association. Diagnostic procedures and reagents. 1st edition, p. 233. A.P.H.A., New York. 1941.
3. BONDE, REINER. Comparative studies of the bacteria associated with potato black-leg and seed-piece decay. Phytopath. 29: 831–851. 1939.
4. BORMAN, EARLE K., C. A. STUART, and KENNETH M. WHEELER. Taxonomy of the family Enterobacteriaceae. Jour. Bact. 48: 351–367. 1944.
5. CAMPBELL, W. A. A bacterial root and stem disease of guayule. Phytopath. 37: 271–277. 1947.
6. Difco Laboratories Incorporated. Manual of dehydrated culture media and reagents. 7th edition. Difco, Detroit. 1943.
7. DOWSON, W. J. On the generic names *Pseudomonas*, *Xanthomonas* and *Bacterium* for certain bacterial plant pathogens. Trans. British Mycol. Soc. 26: 4–14. 1943.
8. ELROD, R. P. The Erwinia-coliform relationship. Jour. Bact. 44: 433–440. 1942.
9. HAGBORG, W. A. F. Classification revision in *Xanthomonas translucens*. Canadian Jour. Res., C, 20: 312–326. 1942.
10. HARDING, H. A., and W. J. MORSE. The bacterial soft rots of certain vegetables. Part I. The mutual relationships of the causal organisms. Vermont Agr. Exp. Sta. Bul. 147: 243–279. 1910.
11. KAHLFELD, F., und A. WAHLICH. Bacteriologische Nährboden-Technik. 2nd edition. pp. 78–79. H. Kornfeld, Berlin. 1929.
12. McCULLOCH, LUCIA. A bacterial leaf spot of horse-radish caused by *Bacterium campestre* var. *armoraciae* n. var. Jour. Agr. Res. [U.S.] 38: 269–287. 1929.
13. Pathology Division, Special Guayule Research Project, U. S. Department of Agriculture. Guayule nursery diseases and their control. 20 pp. 1944. (Mimeographed.)
14. SNYDER, W. C., and H. N. HANSEN. The species concept in *Fusarium*. Amer. Jour. Bot. 27: 64–67. 1940.
15. Society of American Bacteriologists, Committee on Bacteriological Technic. Manual of methods for pure culture study of bacteria. Biotech Publications, Geneva. 1923 to 1946.
16. STARR, MORTIMER P. Studies on phytopathogenic bacteria. Cornell University Abstracts of Theses for 1943, pp. 349–350. 1944.
17. STARR, MORTIMER P. The nutrition of phytopathogenic bacteria. I. Minimal nutritive requirements of the genus *Xanthomonas*. Jour. Bact. 51: 131–143. 1946.
18. STUART, C. A., K. M. WHEELER, ROBERT RUSTIGIAN, and ALICE ZIMMERMAN. Biochemical and antigenic relationships of the paracolon bacteria. Jour. Bact. 45: 101–119. 1943.
19. VAN NIEL, C. B. The culture, general physiology, morphology, and classification of the non-sulfur purple and brown bacteria. Bact. Revs. 8: 1–118. 1944.
20. WALDEE, E. L. Comparative studies of some peritrichous phytopathogenic bacteria. Iowa State College Jour. Sci. 19: 435–484. 1945.

A WILT-INDUCING TOXIC SUBSTANCE FROM CROWN-GALL BACTERIA[1]

ROLAND HODGSON, A. J. RIKER, AND W. H. PETERSON

INTRODUCTION

Investigators of crown gall have employed various techniques for introducing into the plant certain preparations from cultures of the causal bacterium. The crown-gall studies have been reviewed by Riker and Berge (32), Levine (21), and Riker (31). Extracts of whole cultures, of cells, and of culture filtrates have been applied directly, or mixed with a carrier such as lanolin, to the plant with or without wounding (6, 22). Some samples were injected (5). A technique common with investigations on wilt-inducing microorganisms (but apparently not employed previously on crown-gall bacterial filtrates) is to immerse the cut end of a plant shoot in a solution of the culture filtrate. Tomato cuttings were tested in this manner. After they had stood 48 hours in a cell-free filtrate from a crown-gall bacterial culture, the leaflets showed a severe wilting followed by necrosis. Since some metabolite presumably caused these effects, it was sought by fractionation.

The symptoms induced by these filtrates were similar to some of those reported for wilt-inducing microorganisms. The extensive literature about wilting has been reviewed most recently by Brown (7), Harris (13), and Gottlieb (11).

Various theories have arisen concerning the wilting mechanism involved when the wilting symptoms could not be ascribed to tissue disintegration. Mechanical plugging of the plants' conducting vessels by bacteria and their products (36) or by fungal mycelia (35) was early cited as the cause of wilt.

Later investigators gave other explanations. Tochinai (37) suggested that the microorganism growing in the vascular system formed gas emboli, which blocked the transpiration stream. Hutchinson (20) considered that wilting was caused by toxic substances elaborated by the wilt organisms. This toxic-filtrate theory has received considerable support, e.g., recently from Wellman (38), Gottlieb (10, 11), Clauson-Kaas, Plattner, and Gäumann (8), and Plattner and Clauson-Kaas (28, 29).

Chemical studies of such toxic filtrates are relatively few. Several workers have obtained alcohol precipitates that were toxic. Certain of these were thermostable (2, 27) while others were thermolabile (3, 12, 39). The character of such precipitates generally was not investigated. Luz (25) attributed the wilting activity of a Fusarium filtrate to ammonia, which also

was reported by others who did not find a sufficient amount to account for the toxic effects (9).

The association of amino compounds with the main toxic fractions of fungus filtrates was suggested by Liesau (23). Various amines, amino acids, and other nitrogenous compounds were tested by Schaffnit and Lüdtke (34), Ahmet (1), and Lüdtke and Achmed (24). Aqueous solutions (0.5 per cent concentration) of certain of these, *e.g.*, creatine, putrescine di-hydrochloride, colamine, and others, had wilting activity similar to that induced by toxic preparations from Fusarium cultures. Although these workers obtained concentrates of the toxic material, isolation and character-ization of any compounds were not reported. More recently Clauson-Kaas, Plattner, and Gäumann (8) reported the isolation of a wilt-inducing meta-bolic product from old cultures of *Fusarium lycopersici* Sacc. This substance was named lyco-marasmin (28, 29) and was a low molecular weight peptide having an empirical formula of $C_9H_{15}O_7N_3$. Upon hydrolysis it yielded ammonia, glycine, aspartic acid, and a product that appeared to be pyruvic acid. A trace of ferric chloride greatly enhanced the activity of this com-pound.

The purpose of the present paper is to describe a method of measuring the toxic substance produced in cultures of the crown-gall organism and to describe its concentration and chemical properties. An abstract of this work has already appeared (17).

EXPERIMENTAL WORK
Materials and Methods

Cultures and medium.—Two strains of *Phytomonas tumefaciens* (Smith and Townsend) Bergey *et al.* were employed. Both the virulent strain, A6 (40), and the attenuated daughter strain, A6-6 (16), were progenies of single cells. The activity of these cultures was checked at intervals by inocu-lations into tomato plants. In all tests the virulent strain induced galls while the attenuated strain produced only rudimentary or no galls.

The sucrose, urea, mineral-salts medium and the methods employed in growing the bacteria were the same as those described earlier (26), except in some cases the incubation time was longer. All cultures were incubated in liter flasks at approximately 26° C. Aeration was provided by placing the flasks in a shaker having a 5-inch reciprocating stroke and running at 54 strokes per minute. Each flask contained 200 ml. of medium and was provided with a 6-inch extension neck to prevent wetting of the cover. In-stead of cotton plugs, a double layer of 6-ply, oil-treated air filter tissue was used to prevent contamination and to provide increased and uniform entrance of air.

Analytical methods.—Reducing sugar was determined (after hydrolysis in the case of sucrose or polysaccharide) according to the method of Shaffer and Somogyi (33) (Reagent 50 with 5 g. of potassium iodide per liter). Determinations of pH were made with the Beckman pH meter. Osmotic

pressure was measured by the cryoscopic method according to Harris and Gortner (14).

Plant Assay

To aid the chemical study a suitable method was developed for estimating the potency of filtrates, extracts, and concentrates.

Neither filtering nor autoclaving (solution near neutrality) appreciably influenced the wilt-inducing activity of filtrates. Thus, filtrates free from viable cells were prepared from liquid cultures of the crown-gall organism (a) by filtering through a Seitz or a sintered-glass filter or (b) by filtering through a layer of asbestos and Hyflo Super-Cel followed by autoclaving at 15 lb. steam pressure for 20 minutes.

Bonny Best tomato plants for the assay were grown in the greenhouse. In 10 to 14 days after seeding, they were well spaced as they were transplanted into a flat of composted soil containing approximately 25 per cent sand. The plants were used after 2 or 3 weeks when they were 12 to 18 inches in height. Each plant was selected for uniformity of size and leaf area; the upper stem bearing 2 or 3 well-developed leaves was cut under water and left standing in tap water until transferred into the test solution.

The tests were made in a small basement room without windows. Light from fluorescent lamps (three 30-watt bulbs at approximately 30 inches) shone on the cuttings for 18 hours each day. The temperature was 25° C. ($\pm 2°$) and the relative humidity was 70 per cent (± 10 per cent).

The cuttings remained in tap water about an hour before they were transferred into graduated 10-ml. vials containing the test solutions. Graduated 25-ml. test tubes also were employed satisfactorily. Either the volume of the test solution or the weight of the solution and vial was noted before transferring the cuttings. After 46 to 50 hours the amount of liquid absorbed and the degree of wilting were recorded. In some tests the loss in weight of solution from the vials (i.e., the amount taken up by the cutting) was obtained to the nearest 0.01 g., while in others the loss in volume was read to the nearest 0.1 ml. Correction was made for the amount of moisture lost by evaporation from control vials containing no plant cuttings.

The progressive symptoms began with tips of the leaflets becoming wilted and tending to cling to any object touched. Later, margins of the leaflets became curled or rolled. The injury gradually spread to the remainder of the leaflets, while the parts first affected became shriveled and necrotic (Fig. 1). In severe cases the entire leaflets became shriveled, dried, and dead. Successive stages are drawn in figure 2. The stems and main petioles, however, remained turgid although epinasty was apparent in some cuttings. Small, irregular, dark, necrotic areas sometimes appeared on the leaves. The portions of the stem below the surface of the liquid in some cases became softened.

In this paper, such symptoms are referred to broadly as "toxic effects," "injury," or "wilting." "Wilting" has been used frequently as synony-

mous with "toxic effect" by several authors to describe symptoms induced by filtrates from various organisms (19, 34, 39).

The degree of injury or "toxic index" was recorded as 0 to 5 according to severity, as shown in figure 2. Intermediates were sometimes recorded as 1.5, 2.5, ...

FIG. 1. Left: tomato cutting in filtrate from crown-gall bacteria showing a severe wilting and necrosis of the leaflets (toxic index of 4) after 48 hours at 27° C. and at a relative humidity of 80 per cent. Right: tomato cutting in control medium showing no wilting (toxic index of 0).
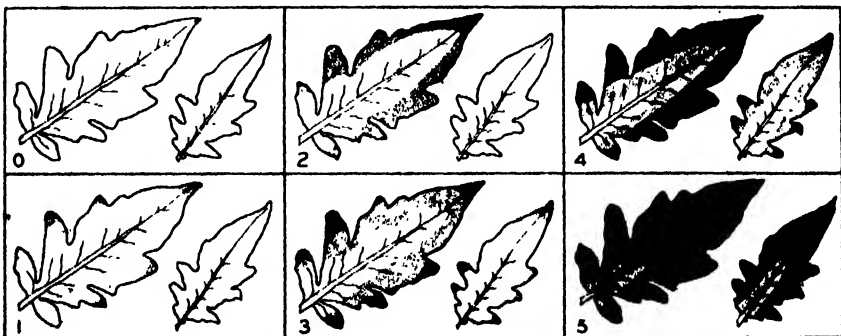
FIG. 2. Method of recording toxic indexes. The small leaf represents an upper and younger leaflet, and the large one represents an older and lower leaflet. Shaded areas represent wilted portions; blackened areas represent necrotic and collapsed portions. 0, no apparent symptoms. 1, wilting is just apparent on the tips of the lower and larger leaflets. 2, edges, as well as the tips of the larger leaflets, are wilted and curled. 3, tips, edges, and part of the inner area of the lower leaflets are severely wilted and curled, and the tips are necrotic; wilting is beginning on the upper leaflets. 4, lower leaflets are curled, necrotic, and collapsed; edges of upper leaflets are wilted and curled. 5, all leaflets are collapsed and necrotic; some may be shriveled and dried; stems are turgid.

Factors affecting the assay.—Two tests on the effect of temperature suggested that the toxic index increased with higher temperatures. Solution intake was greater at higher temperatures with unfermented medium but not with the filtrates. This corresponds with Haymaker's (15) report that tomato plants, whose cut ends were immersed in extracts from *Fusarium lycopersici*, showed symptoms more quickly at higher temperatures and lower humidities.

The effect of pH was studied in 4 trials. The toxic index of plants in filtrates from either the attenuated or the virulent organism was approximately the same at pH levels of 3.0, 5.0, or 7.0. Therefore, in subsequent experiments, some samples were adjusted to pH 5.0, but most were adjusted to pH 3.0 before assay in order to retard growth of contaminating microorganisms. Cuttings in control medium (sucrose, urea, mineral-salts) adjusted to pH 3.0 or 5.0 did not wilt.

In relation to time, readings were taken on many samples at both 24 and 48 hours. A representative comparison of these readings is given in table 1.

TABLE 1.—*Effect of time on solution intake and toxic index of cuttings in filtrates from crown-gall bacteria*[a]

Dilution	Solution intake after		Toxic index after	
	24 hours	48 hours	24 hours	48 hours
	Ml.	*Ml.*		
1.25	2.6	3.1	2.0	3.5
2.5	4.0	5.1	1.1	3.7
5.0	6.3	8.7	0.6	2.3
10	5.0	7.0	0.0	2.2
20	6.3	9.1	0.0	0.8
40	6.6	10.1	0.0	0.5
Unfermented medium	4.3	6.0	0.0	0.0

[a] Samples were adjusted to pH 5.0. Each figure is the average of 10 cuttings.

Both severity of injury and solution intake increased with time. After 24 hours, cuttings in the more dilute solutions showed no or only slight symptoms, whereas after 48 hours these were mildly or severely injured. Therefore, the 48-hour period seemed preferable and was used in subsequent tests.

For comparison, the unfermented medium was tested and had no toxicity (Table 1). A greater volume of this solution was taken in than with the slightly diluted filtrate, but less than with the more diluted sample. The lower intake of unfermented medium probably is related to its higher content of sucrose and salts. Intake of tap water or distilled water always was greater than that of either the filtrate or unfermented medium. Osmotic pressure (14) could not have been the determining factor in these solution intakes as the unfermented medium and filtrate measured 2.67 and 1.06 atmospheres, respectively. The osmotic pressure of pure distilled water is, of course, 0.

Reproducibility of assay values.—Repeated determinations on a given sample were made. Since filtering and autoclaving did not reduce activity, an 8-day-old culture of the virulent organism was filtered through a mat of Hyflo Super-Cel and asbestos; the filtrate was divided into several 100-ml. lots and was autoclaved for 20 minutes at 15 lb. pressure. During 3 weeks samples were assayed 4 separate times, and each experiment was set up at 6 dilutions with 10 plants at each dilution. Average values for the volume of the solution taken up and the toxic indexes recorded after 48 hours are given in figure 3, and the various relationships are indicated. The plants in the stronger solutions showed a greater degree of wilting for the amounts of liquid taken in than the plants in weaker solutions.

The data from each series were analyzed statistically[2] (Table 2). The differences between dilutions for toxic index and for solution intake were

TABLE 2.—*Analysis of variance of the data shown graphically in figure 2*

Source of variance	Degrees of freedom	Mean squares of	
		Solution intake	Toxic index
Dilution	5	342.01[a]	63.24[a]
Linear component	1	1,675.40[a]	311.56[a]
Series	3	4.47	7.62[a]
Error (1)[b]	15	7.56	0.88
Error (2)[c]	215	2.69	0.32

[a] These values exceed the 1 per cent level of significance.

[b] Error (1) is the interaction of dilution and series and was used to test the significance of dilution and series.

[c] Error (2) is the random variation within dilutions within series.

highly significant. Although an analysis of co-variance indicated a straight-line relationship between solution intake and toxic index at the respective dilutions, no close relation existed between toxic index and solution intake within a given dilution (*i.e.*, actual readings in a given trial hit above and below the line). An analysis of variance, considering each series as one replicate, indicated highly significant *F* values between dilutions for both toxicity and solution intake. The differences between series were not significant for solution intake but were highly significant for toxic index. This indicated that the method of estimating toxic index (visual judgment) was less precise than that of determining solution intake. Also plants may be more sensitive at one time than another.

The product of the toxic index and dilution when divided by the solution intake yielded a value that was fairly constant at several dilutions. This value was considered as the number of "toxic units" per milliliter in the original solution. Thus, when assayed at a dilution of 1 to 10, a solution that induced a toxic index of 2.2 and a water intake of 10 ml. would have 2.2 toxic units per milliliter; a concentrate that induced a toxic index of 5 and a water intake of 0.5 ml. at a dilution of 1 g. per 100 ml. would contain 1000

toxic units per gram. Thus, a toxic unit is the amount of substance con-
tained in 1 ml. of a solution that caused a toxic index of 1.

This method of calculation was applied to the data from which those
given in figure 3 were derived, and the toxic unit values (Table 3) were in
fair agreement over the concentration range tested. The precision of the
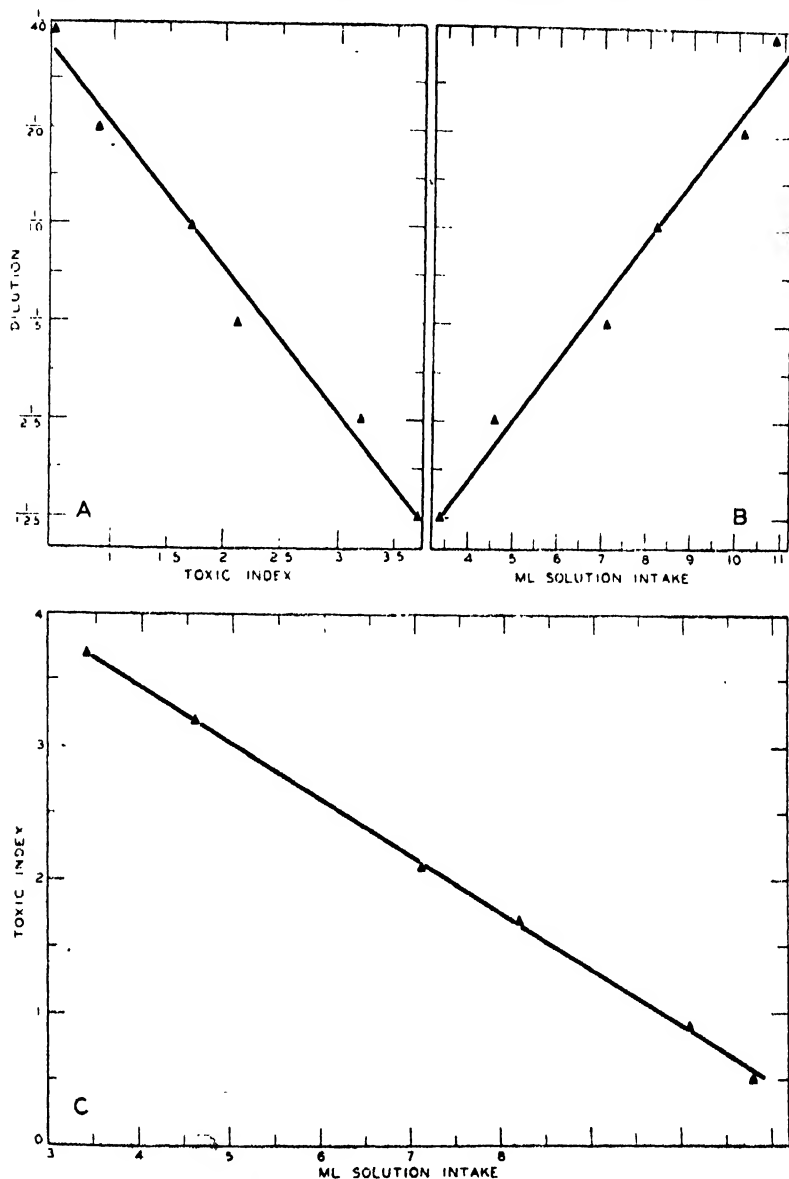
FIG. 3. Relationships of sample dilution, toxic index, and solution intake of tomato
cuttings after 48 hours in a filtrate from crown-gall bacteria at 27° C. and 70 per cent
relative humidity. Each point is the average of 40 cuttings. A, toxic index and dilu-
tions; B, solution intake and dilution; C, toxic index and solution intake.

assay thus appeared such that the method could be used for a semi-quantitative test. The largest variation (excluding the 0 value) between 0.8 and 3.1 was almost fourfold. Larger variations could be expected with samples replicated with fewer than 10 cuttings. Although control of all other factors influencing the plant assay, such as uniformity of size and leaf area of the test cuttings, was limited, thereby making small differences between toxic unit values difficult to interpret, the method was a useful tool in the chemical investigation.

Specificity tests.--The wilting effects described were not specific for filtrates of virulent crown-gall bacteria, since filtrates from the attenuated crown-gall organism (A6–6) also induced similar symptoms. In preliminary tests, few differences, if any, were noted between the filtrate potencies

TABLE 3.—*Toxic units in a fermented eight-day-old culture of virulent crown-gall bacteria*[a]

Dilution	Series number				Average
	1	2	3	4	
	Units per ml.	Units per ml.	Units per ml.	Units per ml.	Units per ml.
1.25	1.4	1.2	1.7	1.3	1.4
2.5	1.8	1.6	1.7	1.6	1.7
5.0	1.3	1.1	2.0	1.4	1.7
10	3.1	1.3	2.2	1.9	2.2
20	1.8	0.8	2.3	1.9	1.7
40	2.0	0.0	3.0	2.4	1.8
Average	1.9	1.0	2.3	1.7	

[a] Calculated from the data shown graphically in figure 2. Each figure represents the average of 10 cuttings.

of the virulent and the attenuated organisms. The details are omitted for the sake of brevity.

The filtrate from crown-gall bacteria induced wilting of cuttings from other plant species, e.g., sunflower, marigold, and pea. This lack of specificity has been noted also for filtrates from various species of *Fusarium* and other microorganisms, which were harmful to a wide variety of plants (2, 4, 12, 34).

Other types of wilting were encountered at various times. Stems of cuttings placed in 0.4 per cent egg albumin suspension wilted in a few hours; wilting of the leaflets followed. Inorganic salts, such as sodium chloride and ammonium sulphate, were tolerated in small concentrations, but in larger concentrations they induced a collapse of the stems that was followed by wilting of the leaflets. Concentrations of sodium chloride less than 0.75 per cent did not induce wilting. However, 0.75 to 1.0 per cent of the salt caused symptoms in some, but not all, of the plants tested, and 1.5 per cent or a higher concentration was very harmful. Plants tolerated concentrations of ammonium sulphate of 0.5 per cent or less, but concentrations of 1 per cent or higher induced similar symptoms.

On several occasions after 70 to 80 cuttings were prepared for an assay, it was noted that both leaflets and stems of 2 to 5 of these sometimes became mildly flaccid, even while standing in tap water. However, these soon recovered when allowed to remain in tap water, and they then appeared normal for several days. This wilting was probably due to the interference with the transpiration stream upon severance of the stem.

Recovery after wilting.—Hursh (19) discussed the importance of testing whether cuttings wilted in the so-called toxic filtrates would recover when a little of the stem was clipped off and the remaining portion was transferred to fresh water. This test was applied to cuttings in filtrates from crown-gall bacteria. After 24 hours in the filtrate the cuttings were mildly wilted; some were transferred to tap water after ¼ inch of the base of the stem was cut off under water. After these cuttings had remained 24 hours in water, the size and appearance of the wilted, curled, and necrotic areas of the leaflets remained about the same, with no increase or decrease. Since there was no recovery, it is apparent that either the injured tissues were too far gone to recover or that the toxic agent was still present and active. The uninjured portions remained green and turgid. This suggested that wilting induced by filtrates from crown-gall bacteria was not due to a mechanical plugging of the vessels at the base of the stem. This idea was supported by the fact that only the leaflets were wilted by the filtrates from crown-gall bacteria, while the stems and petioles remained turgid. However, the possibility of a mechanical plugging in the leaflets remained.

Cuttings in egg albumin solution (0.4 per cent powder) were tested in a similar manner. In 24 hours both stems and leaves were severely wilted. About ¼ inch of the stem's base was cut off under water and some of the cuttings were placed in tap water. After another 24 hours these appeared normal, while those remaining in the albumin were extremely wilted. Probably the albumin plugged the vessels at the base of the stem.

The Nature of the Wilt-Inducing Substance

In each of the tests described below filtrates or concentrates were treated in various ways. They were then assayed to determine any change in their wilt-inducing properties. Unless otherwise stated, all experiments were performed at least two times.

Toxic substance and length of fermentation.—Cultures of the virulent organism, A6, grown in the sucrose, urea, mineral-salts medium incubated for 0, 5, 10, and 15 days were filtered through a mat of asbestos and Hyflo Super-Cel and the filtrates assayed at 6 dilutions (Table 4). The amount of toxic substance in culture increased markedly during the first 5 days. However, the increments thereafter were not statistically significant. Thus, for later tests, cultures 5 to 8 days of age usually were employed.

Fractionation in alcohol.—Several fractions were obtained by treating the fermented media with alcohol by essentially the same procedure used for the separation of the polysaccharide studied earlier (18, 26). For example,

fermented media from two 8-day and one 10-day fermentations were pooled, making a total of 11.5 liters. The liquid was concentrated by distillation under reduced pressure to about 1/30 of the original volume and the concentrate was poured into 1 volume of absolute alcohol. A stringy precipitate of cells and "gum" (Ppt. I) settled at once and was removed by centrifugation. The clear supernatant was concentrated to approximately 200 ml. and was poured into 1.3 volumes of absolute alcohol. A precipitate (Ppt. II) formed and was collected by centrifuging. Ppt. I and Ppt. II were washed in 60 per cent alcohol, and the washings were added to the main supernatant liquid, which then was concentrated to about 200 ml. and was stirred into 2 liters of absolute alcohol. A white precipitate (Ppt. III, lot 1) separated and was allowed to settle for 2 hours or more before it was collected by centrifugation. The supernatant was concentrated to 125 ml. Ppt. III (lot 1) was dissolved in water and reprecipitated in 9 to 10 volumes of absolute alcohol.

TABLE 4.—*The relationship of toxic substance to length of fermentation*

Dilution	Length of fermentation (days)			
	0	5	10	15
	Toxic units per ml.[a]	Toxic units per ml.[a]	Toxic units per ml.[a]	Toxic units per ml.[a]
1.25	0.02	1.1	1.2	1.1
2.5	0.03	1.3	0.9	1.2
5.0	0.05	1.5	1.3	1.4
10	0	1.9	1.4	2.8
20	0	0.4	1.5	0.9
40	0	0.6	0.6	0.9
Averages	0.02	1.1	1.3	1.4

[a] Each figure is the average of 10 cuttings.

For further purification, Ppt. III (lot 1) was triturated in a mortar, first with 90 per cent alcohol, then with absolute alcohol. The washings from each trituration and the supernatant from the final precipitation were concentrated separately. The various precipitates were dried at 60° C. under reduced pressure over sulphuric acid. All fractions were then assayed for toxicity as recorded in table 5.

These data indicated that about 71 per cent of the toxic substance was precipitated in 90 per cent alcohol. However, in a similar fractionation of 2340 ml. of another lot of fermented medium, which was first supercentrifuged to remove bacterial cells and then autoclaved, the precipitate, Ppt. III (lot 2), which separated in 90 per cent alcohol, accounted for only 48 per cent, while the material soluble in 90 per cent alcohol accounted for 50 per cent of the toxic activity.

These rather large differences in the distribution of toxic substance may be related to differences in the amounts formed in the two runs or to variations in the fractionation procedure. In any event, it was clear that the fractions

precipitated by 90 per cent alcohol contained large quantities of the toxic material. These concentrates were stored as dry powders at room temperature for several months with no apparent loss of activity and were convenient for use in subsequent tests.

Nonvolatility.—A solution of the concentrate, Ppt. III (lot 2), was made strongly alkaline with sodium hydroxide and steam distilled; approximately 100 ml. of the distillate were collected. The residue was then acidified with sulphuric acid, again steam distilled, and approximately 100 ml. of distillate were collected. The distillation products showed no toxic activity. Neutral distillation products were not studied separately since they probably would be found with the alkaline distillates which contained no toxic activity. The toxic substance thus appeared nonvolatile.

Parallel results were secured with the attenuated culture.

TABLE 5.—*Distribution of toxic substance in various fractions of fermented crown-gall medium*

Sample	Solubility in alcohol	Total activity per fraction	Weight of solids	Portion of	
				Total solids	Total toxic activity
		Toxic units[a]	*G.*	*Per cent*	*Per cent*
Fermented medium		28,580	115[b]	100	100
Ppt. I	Insoluble in 50 per cent	1,435	15	13	5
Ppt. II	Precipitated in 57 per cent	1,402	6	5	5
Ppt. III (lot 1)	Insoluble in 90 per cent	20,250	72	72	71
Solution IV	Soluble in 90 per cent	4,510			16
	Totals	27,597	93	90	97

[a] Each value is the average of 4 cuttings.
[b] This value was estimated from yields of lyophilized filtrates which were approximately 1 g. per 100 ml.

Dialyzability.—Passage of the toxic agent through membranes was tested by placing 100 ml. of the fermented medium into a Visking cellophane tube closed at the lower end and dialyzing against running tap water. After 4 days the material left in the tube was entirely inactive. It is unlikely that the active substance was destroyed in tap water since it withstood autoclaving at pH 4 to 5.

In another experiment 100 ml. of fermented medium were dialyzed against 2.5 liters of distilled water. After 48 hours both the dialysate and residue were concentrated to 100 ml. and were assayed for toxic activity. Compared to the original fermented medium, which was simultaneously assayed, approximately 50 per cent of the toxic activity was found in the dialysate and 50 per cent in the residue. The toxic substance thus appeared

diffusible through membranes; this suggested that it had a relatively small molecular size.

Solubility.—The toxic substance was very water-soluble. Numerous attempts were made to extract the toxic substance from filtrates and concentrates with various organic solvents. Portions (0.2 g.) of Ppt. III (lot 2) were shaken with 10 ml. of each of the following: methyl alcohol, methyl alcohol made to 1 N acidity with concentrated hydrochloric acid, methyl alcohol-pyridine solution (9 : 1 by volume), methyl alcohol made to 1 N alkalinity with concentrated sodium hydroxide, methyl alcohol-water solution (9 : 1), methyl cellosolve, pyridine, dioxan, acetone, ethyl alcohol, butyl alcohol, benzene, chloroform, ethyl acetate, and petroleum ether. The mixtures were allowed to stand overnight and were filtered. The filtrates were evaporated to dryness on watch glasses. An appreciable amount of residue was left only by acidified methyl alcohol (19 parts methyl alcohol, 1 part concentrated hydrochloric acid), methyl cellosolve, butyl alcohol, and pyridine. Further tests were made with these solvents.

In such cases, 1 g. of the concentrate was triturated with the solvent in a mortar, the mixture was filtered, the filtrate was evaporated to dryness under reduced pressure, and the solid substances were taken up in distilled water. The extracted residue and the portion dissolved with solvent were both assayed for toxic activity. In all cases, the main activity was found in the fraction not soluble in the organic solvent. Similar results were obtained by extracting with ether, butyl alcohol, and benzene from solutions of Ppt. III (lot 2) in 0.1 N sodium hydroxide. Ether extracts from neutral or acidified solutions of Ppt. III (lot 2) or from fermented medium were also nontoxic. The solubility of the toxic substance in the common organic solvents thus appeared slight.

Other tests.—Attempts were made to concentrate and to purify the toxic substance through the use of adsorbents. While the toxic substance was partially adsorbed on Norit, suitable procedures for eluting the substance were not found, and this technique was abandoned.

Procedures with ion exchangers were also employed but were not found satisfactory, since recovery of the adsorbed toxic substance from the ion exchangers was low.

Association of toxic substance and polysaccharide in fractions.—The high toxicity of the preparation precipitated in 90 per cent alcohol and the failure to effect a separation of the toxic substance and the previously studied polysaccharide, which occurs in this fraction (18, 26), suggested an association between toxicity and polysaccharide content. Further evidence of such an association was sought.

The amount of toxic substance and polysaccharide in culture in relation to length of fermentation was studied. Analyses for polysaccharide were made on the cultures described in table 4. The analysis for polysaccharide was made as follows. Samples were analyzed for reducing sugar after hydrolysis in 0.5 N sulphuric acid at 100° C. for 10 minutes and for 2 hours.

Reducing values obtained from samples hydrolyzed 10 minutes were taken
as unfermented sucrose and the difference in reducing sugar between the
10-minute and 2-hour hydrolyses was called polysaccharide. A 10-minute
hydrolysis under these conditions hydrolyzed less than 1 per cent of the poly-
saccharide to reducing sugar.

The data (Table 6) indicated a rapid increase in polysaccharide during
the first 5 days of fermentation and a much slower but definite increase dur-

TABLE 6.—*Sucrose utilization, polysaccharide and toxic substance production, and
length of fermentation*

Length of fermentation	Residual sucrose	Polysaccharide (as sucrose)	Toxic units[a]
Days	*Mg. per ml.*	*Mg. per ml.*	*Per ml.*
0	19.90	0	0.02
5	1.14	3.11	1.1
10	0.32	3.63	1.3
15	0.25	3.91	1.4
20	0.21	4.18	...

[a] These values represent the average toxic units from table 4.

ing the next 15 days. The toxic substance likewise increased rapidly during
the first 5 days but did not increase significantly through the next 10 days.
Thus, there appeared to be a concomitant variation between polysaccharide
and toxicity. Similar evidence was sought with dialysis experiments.

The distribution of toxic substance and polysaccharide upon dialysis of
filtrate from virulent crown-gall bacteria was examined. A cellophane tube
(about 1.1 inches in diameter and 0.00072 inches wall thickness) containing
100 ml. of the filtrate was suspended in a vessel containing 2.5 liters of dis-
tilled water and a few drops of chloroform as a preservative. After 48 hours
both dialysate and residue were concentrated at room temperature under
reduced pressure to 100 ml. Samples of untreated filtrate and the residue
remaining in the tube were analyzed for polysaccharide and then were
assayed for toxic activity. The results (Table 7) indicated that approxi-

TABLE 7.—*Distribution of toxic substance and polysaccharide after dialysis*[a]

Sample	Polysaccharide (as sucrose)	Toxic substance[b]
	Mg. per ml.	*Toxic units per ml.*
Untreated filtrate	5.9	0.8
Residue	2.8	0.4
Dialysate	2.8	0.4

[a] The residue and the dialysate were each concentrated and diluted to the volume of
the untreated filtrate.
[b] Each value is the average of 10 cuttings.

mately one-half of both the toxic activity and the polysaccharide had diffused
through the membrane. Again there appeared to be a quantitative corre-
lation between toxic activity and polysaccharide.

Toxicity of purified bacterial polysaccharide.—Assays of previously puri-
fied polysaccharide preparations indicated that this substance had consider-
able toxic activity. A comparison of the activities of the crude concentrate
and purified polysaccharide is given in table 8.

TABLE 8.—*Toxic activity of the crude concentrate and various purified glucosan
preparations*

Sample	Toxic units
	Per gram
Ppt. III (lot 1) (crude preparation)	766[a]
Preparation No. 2[b]	241[a]
Preparation No. 3	202[a]
Preparation No. 5	379[a]
Preparation No. 6	425[a]
Preparation No. 6 regenerated from the acetate	374[c]

[a] Each value is the average from assays at 2 dilutions, 0.4 and 0.8 g. per 100 ml., with
2 cuttings at each dilution.
[b] Purified glucosan preparations 2, 3, 5, and 6 were the same as those described by
Hodgson, Riker, and Peterson (18) and have low ash and negligible nitrogen contents.
[c] Average value from 2 dilutions, 0.15 and 0.72 g. per 100 ml., with 4 cuttings at each
dilution.

These data indicate greater activities for crude than for purified prepa-
rations. This was so for all tests in which crude and purified glucosan were
assayed simultaneously. However, when all values from separate assays of
Ppt. III (lot 1) were considered, several in the range of those for the purified
preparations were found. Ppt. III (lot 1), assayed on 9 different occasions
at concentrations of 0.4 and 0.8 per cent, had an average activity (28 cut-
tings) of 570 toxic units per gram; the range was 347 to 766. Ppt. III
(lot 2) had an activity of 614 to 745 toxic units per gram in 4 assays at 0.8
to 4 per cent concentrations; the average was 669 (18 cuttings). The range
for purified glucosan (4 preparations) was 202 to 425, and the average was
312 (16 cuttings). (The quantity of highly purified preparations was
limited, hence extensive assays of these materials were not made.)

In order further to determine whether the toxic activity was due to the
polysaccharide itself or to some impurity, a purified preparation of glucosan
(from an attenuated strain, A6–6, of the crown-gall organism) was acetyl-
lated with acetic anhydride and pyridine. The acetylated product, which is
insoluble in water and soluble in chloroform, was purified and its optical
rotation and acetyl value determined. The specific rotation $[\alpha]_D^{20}$ was +59
to +63 ($c = 1$, chloroform), and the acetyl value was 43 per cent. The values
for a purified preparation from a virulent strain, A6, of the crown-gall
organism as given by McIntire, Peterson, and Riker (26) were $[\alpha]_D^{20} = +56$
to +58.5, and the acetyl value was 42 to 44 per cent, respectively. The
acetylated glucosan was then hydrolyzed with 1 N potassium hydroxide in
methyl alcohol, the regenerated polysaccharide was precipitated in 10 vol-
umes of absolute alcohol, collected, dissolved in water, and reprecipitated.
The precipitate was dried under reduced pressure over sulphuric acid at

60° C. and assayed for toxic activity in aqueous solution. Another sample of polysaccharide acetate was hydrolyzed as above. The hydrolysate was passed once through a column of the cation exchanger, Zeo-Karb-H,[3] to remove potassium and then, after the addition of a few drops of dilute sulphuric acid, was concentrated under reduced pressure to remove methyl alcohol and acetic acid. The final solution was diluted with water, adjusted to pH 3 with barium hydroxide, filtered, and assayed for toxic activity. The values obtained for each preparation were, respectively, 383 and 366 toxic units per gram, or an average of 374. A comparison with the original polysaccharide (Table 8) indicated that a slight, but probably not significant, loss in toxic activity had occurred.

Since the polysaccharide maintained its toxic activity through acetylation and regeneration from the acetate and since other tests had failed to separate the toxic activity from the polysaccharide fractions, it appeared that a large portion, at least, of the toxic activity of media fermented by the crown-gall organism was due to the polysaccharide molecule itself.

Additional evidence that the glucosan and the toxic substance are identical is deduced from their similarity of behavior to heating with 1 N hydrochloric acid at 100° C.; in two hours the activity of each is completely destroyed. This treatment completely hydrolyzes the polysaccharide to glucose, which is inactive in the plant test.

DISCUSSION

The foregoing evidence indicates that upon fractionation in alcohol the toxic substance in the culture appears mainly in two fractions; one is soluble in 90 per cent alcohol and the other is insoluble. The fraction precipitated in 90 per cent alcohol is composed largely of glucosan; it is the main toxic component of this fraction. The composition of the fraction soluble in 90 per cent alcohol was not studied critically. However, it might contain small amounts of polysaccharide which were incompletely precipitated in 90 per cent alcohol. In addition, some lower molecular weight glucosans may be present in this fraction.

The variation encountered in plant materials contributed one of the main difficulties when assaying the various fractions for toxic potency. It was deemed necessary to standardize the methods of growing the plants and the temperature and the relative humidity of the test room. The values chosen (25° C. ± 2° and 70 per cent ± 10 per cent relative humidity) were such that cuttings in unfermented medium showed little or no wilting in the 48-hour test period. Even under standardized conditions the variation in toxicity of a given sample tested on different occasions was undesirably large. The toxic values of samples assayed simultaneously were more uniform. Because of previous treatment, some plants appeared to be less sensitive than others to the toxic substance.

A comparison of the toxicity of our preparation with the peptide ob-

tained by the Swiss workers (8, 28, 29) would be of interest, but an adequate comparison is difficult because the two preparations were tested by widely differing techniques and probably under very different conditions of temperature and relative humidity. The Swiss workers used excised leaflets and measured the time to effect a certain degree of wilting as an index of potency, whereas, we employed cuttings bearing 2 to 3 well-developed leaves and recorded the degree of wilting produced in 48 hours. In 30 to 60 hours 2 mg. of their substance plus 0.04 mg. of iron as ferric chloride in 7 ml. of water caused complete wilting of tomato leaflets. Without addition of the ferric chloride 100 to 150 hours were necessary. In our test, an intake of 5 ml. of solution containing 20 mg. of glucosan wilted cuttings to about 70 per cent of the maximum. Thus on the weight basis their preparation seems more toxic. However, when excised leaflets were substituted in our test for cuttings bearing 2 to 3 well-developed leaves, the former appeared to be much more sensitive than the latter. Therefore, any valid comparison of the two compounds cannot be drawn from present information.

The relationship, if any, of the toxic substance to gall formation deserves discussion. First, the question arises whether the toxic substance is produced by the organism when growing in plant tissue since wilting ordinarily is not observed in the crown-gall disease. The lack of wilting may be due to unfavorable conditions in the plant for polysaccharide production and subsequent distribution, e.g., (a) the relatively small numbers of bacteria inside the gall (30), (b) the low concentration of sugar substrate in the plant, and (c) particularly the growth of these bacteria in tissues other than the water-conducting vessels (30) where the so-called wilt organisms grow best. Secondly, filtrates from an attenuated as well as a pathogenic strain of the crown-gall organism induce wilting. (Similar findings for other nonparasitic microorganisms have been reported, e.g., 19, 27.) In the case of the crown-gall organism, the attenuated strain produces the same glucosan as the virulent strain (18). Thus filtrates from the attenuated culture would be expected to induce wilting. On this basis no relationship is at present apparent between the toxic substance and gall formation. However, this toxic substance cannot wisely be ruled out by present information. It is recognized that pathogenicity may depend not on a single character, but on a number of factors which are in suitable balance (31). The final conclusion on this question must await further investigation.

There is no intention to infer that the ability to induce a wilting of plant cuttings is a unique property of the polysaccharide from crown-gall bacteria. Experiments with various other polysaccharides that also induce wilting will be described in a subsequent paper.

SUMMARY

Filtrates from crown-gall bacteria were found to induce in the leaflets of tomato cuttings and other plants a wilting followed by a necrosis. The active (or toxic) substance inducing the wilting was investigated.

Under standardized conditions the approximate quantity of toxic substance in filtrates was measured by an assay involving filtrate concentration, solution intake, and severity of wilting (toxic index). Various factors affected the assay, particularly temperature, humidity, condition of plants, and length of assay period.

The toxic substance was thermostable in neutral solution, labile when heated in strong acid solution, and was nonvolatile, soluble in water, relatively insoluble in most organic solvents and dialyzable.

After alcoholic fractionation of the filtrate, much of the toxic activity appeared in a fraction precipitated in 90 per cent alcohol and consisted largely of a previously studied glucosan.

Several tests indicated a direct concomitant variation between the toxic substance and the polysaccharide. Therefore, several pure preparations of glucosan from crown-gall bacteria were tested and found to induce wilting similar to that of the whole filtrate.

The evidence available points to the glucosan primarily as the toxic substance.

UNIVERSITY OF WISCONSIN,

MADISON, WISCONSIN.

LITERATURE CITED

1. AHMET, H. Untersuchungen über Tracheomykosen. Phytopath. Ztschr. 6: 49–101. 1933.
2. BARNUM, C. C. The production of substances toxic to plants by *Penicillium expansum* Link. Phytopath. 14: 238–243. 1924.
3. BEWLEY, W. F. ''Sleepy Disease'' of the tomato. Ann. Appl. Biol. 9: 116–134. 1922.
4. BISBY, G. R. Studies on Fusarium diseases of potatoes and truck crops in Minnesota. Minn. Agr. Exp. Sta. Bul. 181. 1919.
5. BOIVIN, A., M. MARRE, L. MESROBEANU, and P. JUSTER. Sur l'existence, dans le *Bacillus tumefaciens* d'une endotoxine capable de provoquer la formation de tumeurs chez les végétaux. [Paris] Acad. des Sci. Compt. Rend. 201: 984–986. 1935.
6. BROWN, N. A., and F. E. GARDNER. Galls produced by plant hormones including a hormone extracted from *Bacterium tumefaciens*. Phytopath. 26: 708–713. 1936.
7. BROWN, W. The physiology of host-parasite relations. Bot. Rev. 2: 236–281. 1936.
8. CLAUSON-KAAS, N., P. A. PLATTNER, and E. GAÜMANN. Über ein welkeerzeugendes Stoffwechselprodukt von *Fusarium lycopersici* Sacc. Ber. Schweiz. Bot. Gesell. 54: 523–528. 1944.
9. FAHMY, T. The production by *Fusarium solani* of a toxic excretory substance capable of causing wilting in plants. Phytopath. 13: 543–550. 1923.
10. GOTTLIEB, D. The presence of a toxin in tomato wilt. Phytopath. 33: 126–135. 1943.
11. ————. The mechanism of wilting caused by *Fusarium bulbigenum* var. lycopersici. Phytopath. 34: 41–59. 1944.
12. GROSSMANN, H. Untersuchungen über die Welkekrankheit des Flachses. Phytopath. Ztschr. 7: 545–583. 1934.
13. HARRIS, H. A. Comparative wilt induction by *Erwinia tracheiphila* and *Phytomonas stewarti*. Phytopath. 30: 625–638. 1940.
14. HARRIS, J. A., and R. A. GORTNER. Notes on the calculation of the osmotic pressure of expressed vegetable saps from the depression of the freezing point, with a table for the values of P for $\Delta = 0.001°$ to $\Delta = 2.999°$. Amer. Jour. Bot. 1: 75–78. 1914.
15. HAYMAKER, H. H. Relation of toxic excretory products from two strains of *Fusarium lycopersici* Sacc. to tomato wilt. Jour. Agr. Res. [U. S.] 36: 697–719. 1928.

16. HENDRICKSON, A. A., 1. L. BALDWIN, and A. J. RIKER. Studies on certain physiological characters of *Phytomonas tumefaciens, Phytomonas rhizogenes,* and *Bacillus radiobacter.* Part II. Jour. Bact. 28: 597–618. 1934.

17. HODGSON, R., W. H. PETERSON, and A. J. RIKER. Wilting induced in tomato cuttings by toxic substance from crown-gall bacteria. (Abstr.) Phytopath. 36: 401. 1946.

18. —————— —, A. J. RIKER, and W. H. PETERSON. Polysaccharide production by virulent and attenuated crown-gall bacteria. Jour. Biol. Chem. 158: 89–100. 1945.

19. HURSH, C. R. The reactions of plant stems to fungous products. Phytopath. 18: 603–610. 1928.

20. HUTCHINSON, C. M. Rangpur tobacco wilt. India Dept. Agr. Mem., Bact. Ser. 1: 67–84. 1913.

21. LEVINE, M. Plant tumors and their relation to cancer. Bot. Rev. 2: 439–455. 1936.

22. ——————————, and E. CHARGAFF. The response of plants to chemical fractions of *Bacterium tumefaciens.* Amer. Jour. Bot. 24: 461–472. 1937.

23. LIESAU, O. F. Zur Biologie von *Didymella lycopersici,* dem Erreger der Tomatenkrebskrankheit. Phytopath. Ztschr. 5: 1–40. 1932.

24. LÜDTKE, M., and H. ACHMED. Über einen pflanzlichen Welkstoff. Biochem. Ztschr. 257: 256–266. 1933.

25. LUZ, G. Über den Stoffwechsel von *Fusarium lycopersici* und *Fusarium lini.* Phytopath. Ztschr. 7: 585–638. 1934.

26. MCINTIRE, F. C., W. H. PETERSON, and A. J. RIKER. A polysaccharide produced by the crown-gall organism. Jour. Biol. Chem. 143: 491–496. 1942.

27. PICADO, C. Sur l'action à distance des champignons phytopathogènes. Cong. Path. Vég. Strasbourg, pp. 28–34. 1923.

28. PLATTNER, P. A., and N. CLAUSON-KAAS. Über ein Welkerzeugendes Stoffwechselprodukt von *Fusarium lycopersici* Sacc. Helvetica Chim. Acta 28: 188–195. 1945.

29. ——————————, and ——————————. Über Lyco-marasmin, den Welkstoff aus *Fusarium lycopersici* Sacc. Experimentia 1: 195–196. 1945. (Abstr. in Rev. Appl. Mycol. 25: 84. 1946.)

30. RIKER, A. J. Some relations of the crowngall organism to its host tissue. Jour. Agr. Res. [U. S.] 25: 119–132. 1923.

31. ——————————. The relation of some chemical and physico-chemical factors to the initiation of pathological plant growth. Growth, Fourth Symposium, Sup. to 6, pp. 105–117. 1942.

32. RIKER, A. J., and T. O. BERGE. Atypical and pathological multiplication of cells approached through studies on crown gall. Amer. Jour. Cancer 25: 310–357. 1935.

33. SHAFFER, P. A., and M. SOMOGYI. Copper-iodometric reagents for sugar determination. Jour. Biol. Chem. 100: 695–713. 1933.

34. SHAFFNIT, E., and M. LÜDTKE. Über die Bildung von Toxinen durch vershiedene Pflanzenparasiten. Ber. Deut. Bot. Gesell. 50: 444–463. 1932.

35. SMITH, E. F. Wilt disease of cotton, watermelon, and cowpea. U. S. Dept. Agr. Bul. 17. 1899.

36. STEWART, F. C. A bacterial disease of sweet corn. N. Y. State Agr. Exp. Sta. Bul. 130: 423–439. 1897.

37. TOCHINAI, Y. Comparative studies on the physiology of *Fusarium lini* and *Colletotrichum lini.* Hokkaido Imp. Univ., Faculty Agr. Jour. 14: 171–236. 1926.

38. WELLMAN, F. L. Comparative toxic effects of extracts from mild and virulent isolates of tomato-wilt Fusarium. Phytopath. 33: 1004–1017. 1943.

39. WHITE, R. P. Studies on tomato wilt caused by *Fusarium lycopersici* Sacc. Jour. Agr. Res. [U. S.] 34: 197–239. 1927.

40. WRIGHT, W. H., A. A. HENDRICKSON, and A. J. RIKER. Studies on the progeny of single-cell isolations from the hairy-root and crown-gall organisms. Jour. Agr. Res. [U. S.] 41: 541–547. 1930.

GROWTH AND VARIATION OF SIX PHYSIOLOGIC RACES OF ACTINOMYCES SCABIES ON DIFFERENT CULTURE MEDIA[1]

W. D. THOMAS, JR.

The potato scab organism, *Actinomyces scabies* (Thax.) Güssow, is subject to innumerable chemical and physical influences that may affect the stability of its biotypes and its ability to infect and parasitize its host. Isolated factors that might influence the growth and variation of *A. scabies* on agar media, in soil, and on potatoes were studied. These factors were different carbon sources, fertilizer ratios, fungicides, soil fungus extracts, and soil types.

Six isolates of monosporous lines from mass cultures of original isolations were used. Isolates 11 and 39 originated from type-4 scab pustules[2] on Irish Cobbler potatoes, isolates 46 and 47 from type-1 and type-2 pustules, respectively, on tubers of selection 161.37–29.[3] Isolate 48 came from a type-3 pustule on a tuber of selection 42.32–1–2, while isolate 49 was isolated from a type-2 pustule on a tuber of selection 31.37.[4] These lines differed so decidedly in cultural characters on potato-dextrose agar that they were considered distinct races (Table 1).

TABLE 1.—*Description of six physiologic races of Actinomyces scabies when grown on potato-dextrose agar*

Race	Pigmentation[a] of medium	Color[a] of aerial mycelium	Aerial mycelium	Topography
11	Lemon chrome	White	Abundant	Smooth
39	None	Warm buff	do	do
46	Anthracene purple	Light flesh	do	do
47	Indigo blue	Indigo blue	do	do[b]
48	Dusky brown	White	Sparse	do
49	Light orange yellow	Slate gray	Abundant	do

[a] Pigmentation based on Ridgway (12).
[b] Aerial mycelium in uniform ring-like zones.

[2] Classification of pustules based on that of Leach *et al.* (7).

[3] The numbered potato selections were furnished by Dr. F. A. Krantz of the Division of Horticulture, University of Minnesota.

[4] Isolate 39 originated in sandy soil at Grand Rapids, Minn. The other isolates were obtained from potatoes grown in peat at Coon Creek, near Anoka, Minn.

PATHOGENICITY TESTS

The pathogenicity of the 6 races on 10 potato varieties and selections was determined in the greenhouse. Six-inch pots of sterile peat soil (pH 6.9) were inoculated[5] with individual races one week prior to planting. Six seed tubers of each variety were soaked for 1 hour in 1:1000 mercuric chloride and then washed thoroughly before cutting and planting. Controls for each selection were in sterile, noninoculated peat soil. The temperature of the soil averaged approximately 25° C. during the experiment.

TABLE 2.—*Results of inoculating 10 potato selections with 6 isolates of Actinomyces scabies*

Selection	Infection rating of races[a]						Field reaction[b]
	11	39	46	47	48	49	
26.37–30s	5/2	0/0	0/0	0/0	0/0	0/0	2.0
6–1	0/0	0/0	0/0	0/0	5/2	0/0	1.0
Arnica	0/0	45/1	45/1	0/0	0/0	0/0	0.7
Earlaine	20/3	t/1	80/1	0/0	0/0	0/0	4.0
US41956	45/4	4/54	20/2	5/3	0/0	45/2	4.0
627–240	0/0	5/1	20/1	5/2	0/0	t/1	2.8
528–194	0/0	0/0	0/0	45/1	0/0	t/1	2.1
Hindenburg	t/1	0/0	20/1	0/0	0/0	0/0	1.6
19.37–25s	t/1	t/1	0/0	5/1	20/2	t/1	3.0
26.37–26s	80/3	20/3	45/3	0/0	80/3	45/3	3.0

[a] Prevalence/Severity. Prevalence is given in terms of the percentage of tuber area infected. Severity is given in terms of the mean scab rating. t — trace of infection.

[b] Mean scab type derived by obtaining the mean for the scab readings on individual tubers in each clone from 5-hill units.

Parasitic races of *Actinomyces scabies* have been demonstrated previously by DeBruyn (4), Leach *et al.* (8), and Schaal (13). The reactions of the 10 varieties or selections of potatoes to the 6 races used in the present study differed. Isolates 11, 39, and 46 were the most pathogenic in the greenhouse tests; isolate 48 was severe on selections 6-1, 19.37–25s, and 26.37–26s, and it was the only isolate to infect selection 6–1 (Table 2). Isolates 47 and 49 generally were less virulent than other isolates. From the data it appears that a definite specificity for resistance and virulence exists between host and pathogen.

GROWTH OF RACES ON VARIOUS CULTURE MEDIA

The growth of each race was studied on media containing 11 carbohydrates; 26 combinations of nitrogen, phosphorus, and potassium; 6 fungicides; extracts of 6 soil fungi; and 3 types of soil. The specific substances were added to a suitable basic medium of 1.2 per cent agar or to the modified basic medium of Jensen (6), and the media were adjusted to pH 7.0. The cultures were grown at room temperature. All readings were based on triplicate series of cultures in 250-ml. Erlenmeyer flasks.

Growth on carbohydrate media. Of the carbohydrate media, sucrose,

[5] Inoculum was prepared by washing with 200 ml. sterile water the scab cultures grown on agar slant in 250-ml. bottles.

cellulose, inulin, and maltose were the more favorable for the growth of most races (Table 3). With the exception of race 48, the results on sucrose supported Vaughn's observations (14) that different isolates of *Actinomyces scabies* did not differ in growth on a sucrose medium. According to Afanasiev (1), all parasitic cultures of *Actinomyces* can utilize sucrose.

Vaughn's results (14), showing that physiologic races of *A. scabies* generally grew well on a cellulose medium, are supported by the results of this test. Although no destruction of the cellulose was observed, it is possible that the experiment did not continue long enough to permit this action to become noticeable.

Although Jensen (6) did not find inulin of value for other species of *Actinomyces*, growth of the races in our tests was excellent on this medium. The abundant growth of all but race 48 on maltose medium agrees with observations by Jensen (6) and Waksman (15). Generally the more pathogenic isolates attained the greatest growth on glycerine media; however, race 47, though not exceptionally virulent in greenhouse tests, grew well on glycerine media.

Growth on media containing different NPK ratios. Aerial growth generally was retarded by an increase of nitrogen, phosphorus, and potash salts in the medium (Table 3). Nitrogen alone, or in medium quantities in the presence of small amounts of the other salts, favored the production of aerial mycelium by all races. On the other hand, potash, in excess of the other salts, retarded growth. Phosphorus alone, in excessive amounts, was generally favorable for growth, although some differential reaction between races was observed. Race 49 grew well on nearly all media, apparently because of a comparatively greater tolerance for high salt concentrations.

The differential growth of the races on the various media may indicate the possibility of changing the relative prevalence of individual races in the soil by the use of different fertilizers, dependent on the physiologic races present in the soil. However, no generalization can be made for all races.

Growth on fungicide media. Spergon and Thiosan had some degree of specificity in the inhibition of the growth of the different races (Table 3). Spergon inhibited the more pathogenic races 11 and 39, whereas Thiosan inhibited race 39. The other races had a differential reaction to the fungicides. The ability of race 47 to grow on a 1:10,000 $HgCl_2$ medium was a striking example of specificity, as there was no indication of growth on this medium by the other races. This supports Frutchey and Muncie (5), who found several strains of *Actinomyces scabies* that grew in saturated solutions of mercuric chloride in a tyrosinate liquid medium. However, Lieske (9) reported that aerobic forms of the organism failed to grow on a medium containing a 1:100,000 solution of $HgCl_2$. It is possible that the use of such compounds either as a soil or seed treatment may retard one race more than another.

No growth was observed on media containing 1:1000 solutions of Corona PD-7, Semesan Bel, or yellow mercuric oxide. This substantiates reports by other workers (2, 10).

TABLE 3.—*Growth of 6 physiologic races of Actinomyces scabies on different culture media*

Medium and concentration (g./l.)		Aerial growth ratings of races[a]					
		11	39	46	47	48	49
Carbohydrates[b]							
Levulose	25.5	4	5	5	5	5	3
Galactose	25.5	5	5	5	5	5	4
Mannose	25.5	1	1	2	1	3	1
Soluble starch	26.1	2	1	3	1	4	1
Lactose	22.7	2	1	2	4	3	1
Sucrose	22.9	1	1	1	1	4	1
Dextrose	25.5	1	1	2	2	4	1
Cellulose	26.1	1	1	1	1	4	1
Inulin	20.0	1	1	1	1	1	2
Maltose	22.8	1	1	1	1	4	1
Glycerine	30.8	2	1	3	2	4	3
NPK salts[d]							
(NaNO$_3$, 43 per cent P$_2$O$_5$, KCl)	0–0–0	1	1	1	1	2	1
	1–0–0	1	1	1	1	1	1
	0–1–0	1	1	1	1	3	1
	0–0–1	1	1	5	1	1	1
	1–1–0	1	1	1	1	3	1
	0–1–1	1	1	1	1	1	1
	1–0–1	1	1	1	1	1	1
	1–1–1	1	1	1	1	1	1
	10–1–1	1	1	1	1	1	1
	20–1–1	1	1	1	4	2	1
	1–10–1	1	1	3	1	1	1
	1–20–1	1	1	1	1	2	1
	1–1–10	4	1	1	2	2	1
	1–1–20	3	1	1	3	3	1
	10–10–1	1	3	2	1	4	1
	20–10–1	3	2	1	4	4	1
	10–20–1	3	3	4	4	4	1
	10–1–10	3	2	5	3	3	1
	10–1–20	4	3	2	3	3	3
	1–10–10	3	2	1	3	3	1
	1–10–20	4	3	1	2	4	1
	20–20–1	3	2	2	3	4	1
	20–1–20	4	2	1	4	4	1
	1–20–20	3	3	2	4	4	1
	20–20–20	4	2	2	3	4	4
	10–10–10	3	2	2	1	3	1
Fungicides[e]							
Spergon	1 : 1000	5	5	2	2	2	4
Thiosan	do	4	5	2	3	4	4
Semesan Bel	do	5	5	5	5	5	5
Corona PD–7	do	5	5	5	5	5	5
HgO (yellow)	do	5	5	5	5	5	5
HgCl$_2$	1 : 10,000	5	5	5	3	5	5
Soil fungus extracts[f]							
Melanconium putredinis Wallr.		4	4	4	4	4	4
Aspergillus niger T. & C.		4	4	4	4	2	3
Trichoderma lignorum (Tode) Harz		5	4	4	4	4	5
Penicillium humicola Oudem.		4	4	4	4	4	4
P. digitatum Sacc.		4	4	4	4	5	4
Rhizoctonia solani Kühn		3	4	4	4	4	4
Soil extracts[g]							
Sand		3	3	3	4	4	3
Peat		4	4	2	2	4	4
Clay loam		4	4	4	4	4	4

Growth on media containing extracts of soil fungi. It is known (16) that competition with other micro-organisms limits the development of Actinomycetes in the soil. In this experiment only two of the fungus extracts tested had complete inhibitory effect on the growth of the Actinomycete (Table 3). *Penicillium digitatum* extract inhibited race 48, whereas extracts of *Trichoderma lignorum* inhibited races 11 and 49. The latter results support Daines' observations (3) that *T. lignorum* produces a diffusible substance that is toxic to *Actinomyces scabies*. The other extracts were detrimental to the growth of the isolates except for the effect of *Aspergillus niger* extract on race 48.

Growth on media containing soil extracts. Peat extract favored races 46 and 47 (Table 3), whereas races 11, 39, and 46 grew better on sand extract. It may be possible that the chemical nature of a soil may have a selective effect, allowing some races to grow better than others.

Pigmentation on all media indicated that this character, used by Millard and Burr (11), was a poor criterion for the classification of different species of *Actinomyces*. The extreme variability of this character observed in this investigation seemed to limit its use as a specific character.

VARIATION

Variation among races of *Actinomyces scabies* is common (11, 13), but little is known concerning the factors that may influence the genetic stability of the organism. As nutritional factors might influence the stability of the organism, the frequency of variation and types of variants produced were studied on the media used for studying the characters of growth.

Before the experiments had progressed far it was apparent that certain races often produced variants culturally similar to other variants, both in the same and in other races. Whenever they arose, these variants were constant in their cultural characteristics when transferred to potato-dextrose agar and they were classified into types (Table 4).

Variation on carbohydrate media. Variation was influenced considerably by the carbohydrate media (Table 5). Mannose, starch, and sucrose caused considerable sectoring by all races, yet very little sectoring occurred on the cellulose medium. The low figures for levulose and galactose in the table were due to the absence of growth on these media.

ᵃ Aerial growth: 1, excellent; 2, good; 3, fair; 4, poor; 5, none.
ᵇ Basic medium after Jensen (6), modified: potassium phosphate (KH_2PO_4), 2.0 g.; magnesium sulphate ($MgSO_4$), 0.5 g.; sodium chloride ($NaCl$), 0.5 g.; agar, 12 g.; distilled water, 1000 ml.
ᶜ Cellulose obtained by macerating filter paper and suspending the material in the basic medium.
ᵈ Basic medium 12 g. agar in 1000 ml. distilled water.
ᵉ Basic medium was Jensen's modified medium: salts and agar were added to 1 liter solution of fungicide. Media sterilized intermittently in flowing steam 1 hour for 3 days.
ᶠ Fungi were grown on potato broth which was then filtered under aseptic conditions through a sintered glass bacteriological filter into melted agar. Basic medium: 12 g. agar in 1 liter extract.
ᵍ Basic medium and preparation as for the soil fungus media.

TABLE 4.—*Frequency and cultural characters of variant types produced by 6 physiologic races of Actinomyces scabies*

Variant type	Pigmentation[a]		Aerial growth[c]	Topography[d]	Frequency[e]
	Medium[b]	Mycelium			
1	None	None	−	S	89
2	Apricot yellow	Apricot yellow	−	S	10
3	None	White	+	S	65
4	Salmon orange	Dark gull gray	+	S	12
5	None	Cream	+	R	9
6	do	Lavender gray	+	R	4
7	do	Warm buff	+	S	15
8	do	Ochraceous salmon	−	S	3
9	do	Dark gull gray	+	S	2
10	Dark grayish-olive	do	+	S	1
11	None	Deep gray olive	−	S	12
12	Deep cadet blue	Deep cadet blue	+	S	3
13	None	White	+	S	396
14	do	Neutral red	−	S	2
15	do	Orange chrome	−	S	2
16	Indigo blue	Indigo blue	−	S	9
17	Garnet brown	Violet gray	+	S	13
18	Deep cadet blue	Dark Corinth. purple	+	S	1
19	None	Parula blue	−	S	1
20	Ochraceous buff	Ochraceous buff	−	S	9
21	Dark Corinth. purple	Bordeaux	−	S	5
22	do	Deep cadet blue	+	S	5
23	do	Cream	+	S	8
24	Neutral red	White	+	S	9
25	Indigo blue	Xanthene orange	−	W	1
26	None	Indian lake	−	S	1
27	do	Scarlet	−	S	1
28	Indigo blue	Light cadet blue	+	S	6
29	Dull violet black	Pallid mouse	+	S	17
30	Indigo blue	Vinaceous drab	+	S	7
31	None	Ochraceous buff	−	S	51
32	Blanc's blue	Light cadet blue	+	S	8
33	None	Bluish black	−	S	5
34	do	White	−	S	6
35	do	Colorless	−	R	1
36	do	Cream	−	S	5
37	do	Mummy brown	−	R	1
38	do	Orange	−	S	3
39	do	Lemon yellow	−	R	11
40	Dull violet	Gull gray	∓	S	114
41	None	Lemon yellow	+	S	5
42	Dull violet black	White	+	S	10
43	Scarlet	Lemon yellow	−	R	4
44	do	Gull gray	+	S	63
45	do	White	+	S	124
46	None	Buff yellow	−	S	20
47	do	Bittersweet orange	−	S	11
48	do	Pinard yellow	−	R	13
49	do	Gull gray	+	S	151
50	Pyrite yellow	Pyrite yellow	−	S	8
51	Lemon yellow	Dark gull gray	+	S	1
52	Salmon orange	White	+	S	11
54	None	Lemon chrome	+	S	4
55	Ochraceous buff	Hermosa pink	+	S	9
56	Indigo blue	Indigo blue	+	S	13
57	Eugenia red	Hermosa pink	+	S	43
58	Lemon yellow	Black	+	S	1
59	Indigo blue	Gull gray	+	S	1
60	Chestnut brown	White	+	S	1

TABLE 4.—(Continued)

| Variant type | Pigmentation[a] | | Aerial growth[c] | Topography[d] | Frequency[e] |
	Medium[b]	Mycelium			
61	Lemon yellow	Gull gray	+	S	4
62	Cadmium yellow	Tawny	–	R	1
63	Light orange yellow	Slate gray	+	S	1
64	Dull violet black	Gull gray	+	R	1
65	None	Ochraceous tawny	–	W	1
66	do	Mouse gray	+	S	12
67	Garnet brown	Neutral gray	+	S	53
68	Honey yellow	Smoke gray	+	S	1
69	Neutral red	Deep olive buff	+	S	59
70	Lemon yellow	Pale bluegreen gray	+	S	1
71	Diamin-azo blue	Nigrosin blue	+	S	1[*]
72	Lemon yellow	White	+	S	3
73	Blackish brown	Slate gray	+	S	1
74	Pale flesh	Pale flesh	+	S	10
75	Eugenia red	Gull gray	+	S	25
76	None	Sooty black	–	R	3
77	do	Ivory yellow	+	S	12
78	Dark Varley's gray	French gray	+	S	3
79	Carmine	Eosine pink	+	S	6
80	None	Slate gray	+	S	30
81	Neutral red	Flesh	+	S	32
82	Corinthian purple	Dark gull gray	+	S	11
83	Indian red	Light buff	+	S	135
84	Light orange yellow	Pale pink buff	+	S	16
85	Deep slate olive	White	+	S	3
86	None	Smoke gray	+	S	6
87	Cadmium yellow	Cream buff	+	S	34
88	Bishop's purple	do	+	S	27
89	None	Dark olive gray	+	S	6

[a] Pigmentation is based on Ridgway's "Color Standards and Color Nomenclature" (12).

[b] Pigment diffused into the medium from the colony.

[c] Indicates presence (+) or absence (–) of aerial growth.

[d] S, smooth; R, rough; W, wrinkled.

[e] The total number of times each variant was observed in 918 chances.

Variation on NPK media. The effect of different NPK ratios on the frequency of sectoring was variable (Table 5), and no conclusions could be drawn. However, certain races were less stable on media in which the salts were present in small quantities. In the field this might lead to the formation of new races in the presence of fertilizers used at low concentrations.

Variation on fungicide media. The fungicide media had little effect upon the stability of the organism. Only one sector appeared in race 46 on Thiosan medium (Table 5); no sectors appeared on the other media. Consequently, the use of these compounds may have little influence on the formation of new races in the field.

Variation on media with soil-fungus extracts. Extracts of 6 soil fungi influenced the stability of races 48 and 49. Race 48 produced 2 sectors of variant type 8 on *Aspergillus niger* extract medium (Table 5), and race 49 produced a variant each on *Penicillium digitatum* and *Rhizoctonia solani* extract media. Thus, on extracts of these soil fungi new races arose. Al-

TABLE 5.—*Effect of media on the rate of variation among 6 physiologic races of Actinomyces scabies*

Medium	Variation among races[a]						Total
	11	39	46	47	48	49	
Carbohydrates							
Levulose	0/0	0/0	0/0	0/0	0/0	8/4	8/4
Galactose	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Mannose	0/0	12/1	18/4	2/1	4/4	127/2	156/12
Soluble starch	1/1	7/2	14/6	19/3	9/2	30/4	60/18
Lactose	24/2	27/1	9/3	0/0	10/1	88/2	48/9
Sucrose	1/1	37/2	10/4	10/3	2/1	147/3	207/14
Dextrose	5/3	0/0	0/0	6/2	5/3	4/1	18/11
Cellulose	0/0	0/0	5/1	3/1	0/0	8/1	16/3
Inulin	0/0	5/1	6/1	1/1	5/1	5/2	22/6
Maltose	0/0	11/1	2/1	3/1	2/2	117/2	135/7
Glycerine	0/0	1/1	0/0	3/1	8/1	23/3	35/8
NPK ratios							
0-0-0	0/0	0/0	26/4	6/2	8/1	16/1	56/8
1-0-0	1/3	1/1	9/4	6/2	2/1	0/0	21/9
0-1-0	0/0	1/1	10/1	12/2	0/0	3/2	26/6
0-0-1	0/0	1/1	0/0	11/4	3/1	3/1	18/7
1-1-0	2/2	4/2	28/4	15/1	3/1	15/2	67/12
0-1-1	14/2	1/1	16/2	12/1	11/1	25/2	79/9
1-0-1	22/1	0/0	35/3	0/0	5/1	3/1	65/6
1-1-1	9/1	11/1	11/2	19/2	5/1	0/0	55/7
10-1-1	0/0	0/0	9/1	11/1	2/1	0/0	22/3
20-1-1	0/0	12/1	0/0	0/0	3/1	2/1	17/3
1-10-1	0/0	0/0	0/0	0/0	12/1	0/0	12/1
1-20-1	0/0	0/0	0/0	9/2	0/0	0/0	9/2
1-1-10	22/1	4/1	3/1	22/3	3/1	5/1	59/8
1-1-20	0/0	9/1	0/0	12/1	0/0	0/0	21/2
10-10-1	9/2	3/1	0/0	0/0	0/0	60/1	72/4
20-10-1	0/0	0/0	3/1	0/0	0/0	0/0	3/1
10-20-1	11/2	0/0	0/0	9/1	0/0	0/0	20/3
10-1-10	8/1	8/1	0/0	6/1	0/0	0/0	22/3
10-1-20	8/1	3/1	0/0	3/1	0/0	0/0	14/3
1-10-10	11/1	0/0	4/2	4/1	3/1	0/0	22/5
1-10-20	7/1	0/0	0/0	9/1	0/0	0/0	16/2
20-20-1	0/0	11/1	0/0	9/1	0/0	17/2	37/4
20-1-20	0/0	0/0	5/1	2/1	0/0	33/1	40/3
1-20-20	11/1	11/1	9/1	11/3	0/0	0/0	42/6
20-20-20	7/1	0/0	0/0	2/1	0/0	3/1	12/3
10-10-10	9/1	11/1	3/1	5/1	0/0	8/1	36/5
Fungicides[b]							
Thiosan	0/0	0/0	1/1	0/0	0/0	0/0	1/1
Soil fungus extracts[c]							
Aspergillus niger	0/0	0/0	0/0	0/0	2/1	0/0	2/1
Penicillium digitatum	0/0	0/0	0/0	0/0	0/0	4/1	4/1
Rhizoctonia solani	0/0	0/0	0/0	0/0	0/0	2/1	2/1
Soil extracts							
Sand	9/1	0/0	0/0	0/0	0/0	1/1	10/1
Peat	0/0	0/0	0/0	0/0	0/0	16/1	16/1
Loam	0/0	0/0	0/0	0/0	0/0	24/1	24/1
Totals	111/29	181/25	236/49	232/46	110/30	777/46	1647/238

[a] Total number of sectors/total number of variant types.

[b] No sectors were observed on Spergon, Semesan Bel, Corona PD-7, HgCl$_2$, or HgO media.

[c] No sectors were observed on media containing extracts of *Melanconium putredinis*, *Trichoderma lignorum*, or *Penicillium humicola*.

though these cultural studies are not a definite indication of field reaction, the effect of certain soil fungi on *Actinomyces scabies* in culture emphasizes the importance of considering such a phenomenon in the field.

Variation on soil-extract media and in soils. The rate of mutation of most races was not influenced by extracts from different soils (Table 5); but race 49 was variable on extracts of peat and of clay loam, and race 11, the most stable of all the races studied, produced 9 sectors on sand extract. These results indicated that the chemical nature of the soil may influence the genetic stability of certain races, and such an influence must be considered as a factor in the formation of new races in the field.

Experiments with the different races in soils containing a mixture of the six soil fungi showed that the soil organisms had little effect on the less pathogenic races. The most striking results (Table 6) showed that during one year all races were more stable on peat soil than in sand, on the basis of

TABLE 6.—*The effect of soil organisms on the stability of 6 physiologic races of Actinomyces scabies growing in 2 types of soil*

Series	Amount of variation[a]				Total no. colonies
	On sand after		On peat after		
	2 mo.	12 mo.	2 mo.	12 mo.	
11	0/0	1/45	0/0	0/0	1
11 +[b]	0/0	0/0	0/0	0/0	0
39	0/0	1/45	0/0	0/0	1
39 +	0/0	0/0	1/4	0/0	1
46	0/0	20/45	0/0	2/45	22
46 +	0/0	0/0	0/0	2/45	2
47	0/0	26/45	0/0	3/45	29
47 +	1/5	10/45	0/0	0/0	11
48	0/0	3/66	0/0	1/45	4
48 +	0/0	0/0	0/0	0/0	0
49	2/13	16/13	2/13	2/13	22
49 +	0/0	0/0	1/13	0/0	1
Total no. colonies	3	77	4	10	94

[a] Number of colonies/variant type.
[b] + denotes inoculation of the soil with 6 soil organisms.

colonies present in the dilution plates. Except for variant type 13, the variant types isolated after 2 months usually were not obtained after 12 months. The variant types 4 and 5 were weak variants which failed to grow upon repeated transfers to potato-dextrose agar. The predominance of variant type 45 after 12 months was very striking and is difficult to explain. Stock cultures were carried in peat soil after this test had been made, because the physiologic races seemed to maintain their stability better in peat than on nutrient agar or in sand. This fact may offer an explanation for the variability of scab reaction among different types of soils. The relative stability of the races in the presence of the other soil organisms is not significant, as competition by these soil organisms could have prevented the establishment of many variants in the soil.

Variation on the host.   When the different races of *Actinomyces scabies* were reisolated from the tubers at the conclusion of the pathogenicity tests, several variants appeared in the dilution plates (Table 7).   Variant type 4 was isolated from tubers inoculated with races 11, 47, and 49, respectively.

TABLE 7.—*Variation of 6 races of Actinomyces scabies arising from scab pustules in pathogenicity tests on 7 potato selections*

Potato selections	Variation of races[a]					
	11	39	46	47	48	49
6–1					2/31	
Earlaine			1/17 1/22			
US 41956	4/4			3/4		2/39 2/52
627–240		1/10				
528–194						1/39
19.37–25s	1/4				2/31	1/4 1/39
26.37–26s	3/4				3/39	3/39

[a] Pustule type/variant type.

In all, this variant was isolated 5 times from 3 host selections.   It was not associated with any particular pustule type, being found in a type-1 pustule on selection 19.37-25s, in a type-3 pustule on selection 26.37-26s, and in pustules of type-3 and type-4 on selection US 41956.   These pustule types were characteristic of the reaction of the parent race on each selection; since nothing is known of the pathogenicity of the variants, it was assumed that the symptoms were produced by the parent race.   This is further substantiated by the fact that the parent races were also isolated from the same pustules, and in much greater abundance than were the variants.   It was unlikely that these "variants" were contaminants, because the absence of scab on check plants showed that such contamination was rare.   Apparently variation in the organism while living parasitically on the host is a rather common occurrence.

The results of the different tests described in this paper indicate that the more pathogenic races, 11 and 39, were also the more stable.   Because of the poor growth of race 48, although comparatively few sectors were produced, it was considered less stable.   The other races sectored rather frequently and often produced the same variant types (Table 5), which might indicate a close genetic relationship among races.   Some variant types were predominant in a single race, such as type 83 in race 11 and type 49 in race 49.

DISCUSSION

Growth and genetic stability of physiologic races of *Actinomyces scabies* on agar media are influenced by different balances of carbohydrates and salts.   Such balances, either in the host or in the soil, may possibly influence

the presence of certain races, and the production of new races. The apparent differences in growth among races of various carbohydrates may indicate the ability of some races to survive better as saprophytes than others.

Although many workers have found that fertilizers affect the incidence of scab infection, this study indicates that the ratio between nitrogen, phosphorus, and potash is not consistent in its effect on all physiologic races.

In general, the fungicides tried had no pronounced effect on the genetic stability of the organism, but distinct differential growth reactions were observed on Spergon and Thiosan media. As these two compounds are generally used in seed treatment, because of their specificity toward certain organisms, their specific effects on certain physiologic races of *A. scabies* are consequently of some interest. The ability of race 47 to grow on mercuric chloride media indicated that the use of this compound as a seed disinfectant may not mean necessarily that the organism on the seed tuber will be killed. Under field conditions the occurrence of such a reaction is open to question; however, the possibility of seed treatment fungicides exerting a selective effect on physiologic races of *A. scabies* in the field should be investigated further before there can be assurance that a specific compound can control the disease.

Experiments with other soil fungi in relation with the physiologic races of *A. scabies* showed not only that certain organisms may inhibit or produce a differential effect on the growth of these races on agar media, but also that other organisms may upset the genetic stability of certain races. It seems probable that fluctuations in the incidence of scab in different areas may often be due to the presence of certain microflora which could either favor the growth of some races of the scab organism to the exclusion of others or influence the formation of new races through variation.

Results with soil-extract media and soil types indicated that some races can survive better in one soil than in another. Although the data presented herein are not conclusive, they open an interesting field of investigation. That soil types have a pronounced influence on the genetic stability of the organism is of extreme importance. In many sandy areas the scab reaction on a given potato variety is variable, the pustule types ranging from 1 to 4. On the other hand, in many peat soils scab reaction is more or less constant, with little variation in the pustule type. The effects of soil type may be dependent to some extent on the antagonistic action of the soil microflora on the scab organism.

The variation of the organism while living parasitically on the host is of considerable interest, as it offers another possibility for the introduction of new races of *A. scabies* into the soil. Thus, it is evident that the growth and genetic stability of these races are dependent on a complex interrelationship of physical, chemical, and biological factors. Any deviation of these factors may be capable of favoring the survival of some races to the exclusion of others, or the formation of new races.

SUMMARY

Six physiologic races of *Actinomyces scabies* (Thax.) Güssow were distinctly different in pathogenicity on ten different potato varieties or selections.

Sucrose, cellulose, inulin, and maltose media were the most favorable sources of carbon for the growth of these races.

Increasing amounts of nitrogen, phosphorus, and potash retarded the production of aerial mycelium by most races. Nitrogen and phosphorus were generally favorable for growth; potash tended to retard it.

Spergon and Thiosan, at the concentration of 1:1000, were specific in their effect on the growth of the different races; one race grew on media containing a 1:10,000 dilution of mercuric chloride. No growth was observed on media containing 1:1000 dilutions of Corona PD-7, Semesan Bel, or yellow mercuric oxide.

Trichoderma lignorum extract inhibited the growth of 2 races, and 1 race failed to grow on a medium containing an extract of *Penicillium digitatum*. Other soil organisms studied had no effect on growth.

Maximum growth and stability were observed on peat soil; mineral soils tended to retard or inhibit growth and increase variability in the races studied.

The more pathogenic races were most stable on most media.

Variants were produced by some physiologic races while living parasitically on the host.

Some variant types were peculiar to individual races, but certain types were produced frequently by several races, which seemed to indicate a close genetic relationship between those races.

UNIVERSITY FARM,
    ST. PAUL, MINNESOTA.

LITERATURE CITED

1. AFANASIEV, M. M. Comparative physiology of Actinomyces in relation to potato scab. Nebr. Agr. Exp. Sta., Res. Bul. 92. 1937.
2. CUNNINGHAM, H. S., and P. H. WESSEL. Controlling common scab of the potato on Long Island by the addition of mercury compounds to the fertilizer mixture and the relation of soil reaction to the treatment. N. Y. (Geneva) Agr. Exp. Sta. Bul. 685. 1939.
3. DAINES, R. H. Antagonistic action of Trichoderma on Actinomyces scabies and Rhizoctonia solani. Amer. Potato Jour. 14: 85–93. 1937.
4. DEBRUYN, H. L. G. Onderzoekingen over enkele Actinomyceten welke Aardappel-schurft verwekken. Tijdschr. Pl. Ziekt. 45: 133–156. 1939. Abst. in Rev. Appl. Mycol. 19: 41–42. 1940.
5. FRUTCHEY, C. W., and J. H. MUNCIE. Soil treatment with mercurials for control of potato scab. (Abstr.) Phytopath. 24: 9. 1934.
6. JENSEN, H. L. Actinomycetes in Danish soils. Soil Sci. 30: 59–77. 1930.
7. LEACH, J. G., F. A. KRANTZ, P. DECKER, and H. MATTSON. The measurement and inheritance of scab resistance in selfed and hybrid progenies of potatoes. Jour. Agr. Res. [U.S.] 56: 843–853. 1938.
8. ————, P. DECKER, and H. BECKER. Pathogenic races of Actinomyces scabies in relation to scab resistance. Phytopath. 29: 204–209. 1939.
9. LIESKE, R. Morphologie und Biologie der Strahlenpilze (Actinomyceten). Leipzig. 292 pp. 1921.

10. MARTIN, W. H. Potato scab control with organic mercury compounds. Proc. 13th Ann. Meeting, Potato Assn. of America, 1926. pp. 74–81. 1927.
11. MILLARD, W. A., and S. BURR. A study of twenty-four strains of Actinomyces and their relation to the types of common scab of potato. Ann. Appl. Biol. **13**: 580–644. 1926.
12. RIDGWAY, R. Color standards and color nomenclature. Washington. 43 pp., illus. 1912.
13. SCHAAL, L. A. Variation and physiologic specialization in the common scab fungus (Actinomyces scabies). Jour. Agr. Res. [U.S.] **69**: 169–186. 1944.
14. VAUGHN, J. R. Factors affecting the nature of resistance of potatoes to scab. M.S. Thesis, University of Minnesota. (Unpublished.) 1940.
15. WAKSMAN, S. A. Studies in the metabolism of Actinomyces. II. Jour. Bact. **4**: 307–330. 1919.
16. WIERINGA, K. T., and L. W. WIEBOLS. De Aardappelschurft en de heterolyse der schurftparasiet. Tijdschr. Pl. Ziekt. **42**: 235–240. 1936. Abst. in Rev. Appl. Mycol. **16**: 121. 1937.

RESISTANCE TO MEALYBUG WILT OF PINEAPPLE WITH SPECIAL REFERENCE TO A CAYENNE-QUEEN HYBRID[1]

WALTER CARTER AND J. L. COLLINS[2]

INTRODUCTION

The control of mealybug wilt can be considered the major biological problem with which pineapple growers in Hawaii are concerned. Direct control measures against the insect [*Pseudococcus brevipes* (Ckl.)] have achieved a practical degree of success but only at great and continued expense. The development of a resistant variety, even if only moderately resistant, would be of value, either in reducing the cost of current control measures, or in improving the degree of control obtained at present. Testing of pineapple varieties indicated very early that there were varietal differences in susceptibility to mealybug wilt. Since then a number of varietal hybrid populations have been tested for susceptibility to wilt. From these tests it is now possible to report progress, first in establishing that resistance occurs, second in indicating something of the genetic aspect of the resistant character, and finally to reveal some of the unique aspects of the problem with which future studies must be concerned.

PINEAPPLE VARIETIES USED IN HYBRIDIZATION

Eleven varieties of pineapples (Table 1) were studied with respect to susceptibility to mealybug wilt. These were collected from widely separated geographical areas and little is known of their origin or genetic relationships. Some of them can be placed into related groups on the basis of generally similar characters.

Cayenne is the variety grown commercially in Hawaii and a number of other countries, and is highly susceptible to mealybug wilt. Sarawak appears to be a strain of the Cayenne variety which had been grown in the province of Sarawak in northeast Borneo, and it is morphologically indistinguishable from Cayenne. Queen, a spiny-leaved, small-fruited variety grown in a number of countries, is perhaps one of the oldest named varieties of pineapple. Natal and MacGregor closely resemble Queen in their general appearance. Red Spanish is grown commercially in Florida and the West Indies where it has been considered more hardy and vigorous than Cayenne. Pernambuco is a hardy, spiny-leaved form grown in some parts of Brazil. Monte Lirio is a smooth-leaved, white-fleshed variety grown to some extent

in Mexico and Central America, but it lacks growth vigor in Hawaii. Ruby is a smooth-leaved variety from British Malaya where it has been one of several cultivated forms. *Ananas ananassoides* is a hardy, small-fruited, thorny-leaved wild species distributed over a wide area in the dry regions of Brazil. Congo is a spiny-leaved, small-fruited variety from the Belgian Congo region of Africa and it has no characters of commercial value in Hawaii.

The resistant hybrid No. 7898 with which this report specifically deals was selected in 1928 from a Cayenne × Queen F_1 population. It is, in general, a mosaic of the characters of the two parents, with dominance of the Queen variety showing in fruit color and texture and in type of slips and suckers, but in fruit size it is more like Cayenne. The leaves of this hybrid, No. 7898, normally have less anthocyanin pigment than does the Cayenne parent. The chlorophyll color of the leaves is a light pea green in contrast to the darker sage green of the Cayenne variety. The hybrid also ripens its fruit from two to three weeks earlier than its Cayenne parent. Cayenne normally produces 1 to 2 suckers which produce the ratoon crop while the hybrid produces from 3 to 6 suckers.

METHODS OF TESTING FOR RESISTANCE

The several methods used consisted essentially of exposing the plants to mealybug infestation. The first was designed to provide as equal an infestation as possible for each plant tested. In this method, mealybugs were collected from wilting areas in pineapple fields and the medium-sized specimens separated out.[3] These were accumulated *en masse*, and then lots of 50 were enclosed in vials and carried out to the field plots the following day, the insects being without food in the interim.

A second method was to use the rinds of heavily infested green fruits. These were collected one day and carried to the field the next, the pieces of rind bearing mealybugs being cut off just prior to depositing them on the plants to be infested. Since this method has been used also when great numbers of plants were to be infested, the fruits were cut and the pieces removed to the field plots in buckets. One piece was applied to the center of each plant. This method is useful where large numbers of plants are to be grossly infested, but the number of mealybugs actually applied by this method is often very large, and since the bugs must move from the fruit tissue as it dries up, there often is also movement away from the infested plant onto nearby checks.

A third method, much more severe than either of the others, consists of

---

[3] The term "wilting area" means a section of a pineapple field, usually on a border, where wilted plants are common, or predominant. The largest mealybug colonies are found in such circumstances on the apparently healthy plants, and it is from these that the mealybug-infested fruits are usually taken. If fruits on wilted plants are selected, colonies are usually smaller, and experience has shown that these colonies are no more efficient in inducing wilt than are those from nearby healthy plants; on the contrary some large-scale infestations made from mealybugs from these wilted plants have induced a disappointingly small incidence of wilt when transferred to test plants.

growing the plants in an area where natural infestation can occur and be left untouched by artificial control methods. Biological factors cannot of course be controlled, but in the presence of the ant, *Pheidole megacephala* Fab., these factors are seldom sufficient to prevent the maintenance of a mealybug colony.

A fourth method, the one most recently used, is to permit the establishment and maintenance of mealybugs on the variety and to make adjacent new plantings so that young plants could be infested naturally from the older infested planting of the same variety. Here the new plantings are infested with bugs which had been grown on the same variety. In the third method, the bugs which infest the new planting may have been developed upon a different variety.

THE SUSCEPTIBILITY OF CAYENNE WHEN COMPARED WITH OTHER VARIETIES AND HYBRIDS

This was originally determined by the first method of testing, namely, to infest each plant with 50 mealybugs and to permit the infestation to operate only for about two weeks. It is clear from the results in table 1, that Cayenne is the most susceptible variety to be included in these tests. This finding is in accord with experience in other parts of the world where other varieties have replaced Cayenne as the commercial varieties, because of the susceptibility of Cayenne to mealybug wilt (2).

The susceptibility of variety hybrids was also determined by the first method of testing. From the results shown in table 2, it is evident that susceptibility is a dominant character in F_1 hybrids. As with Cayenne, there is considerable variation in the number of plants wilting following specific infestations (1, 4), and for that reason the data are arranged to show the percentage of hybrid plants wilting with an infestation which at the same time induced a given percentage of wilt in Cayenne.

EXPERIMENTS WITH HYBRID NO. 7898 IN PLANTATION FIELDS

In these experiments No. 7898 was planted alongside Cayenne in plantation fields along borders where natural infestation was most likely to be

TABLE 1.—*Tests with pineapple varieties. Wilt following single infestations of 20 plants of each variety with 50 mealybugs per plant*

Variety	Percentage of plants wilted when Cayenne wilted as follows:				
	16	30	40	50	60
Red Spanish	0	30		0	
Ananas ananassoides	0				
Pernambucó	10	0	0		15
Queen	2	0	0	0	10
Sarawak					0
Congo			5		5
Natal			0		0
MacGregor					20
Ruby					0
Monte Lirio				5	

TABLE 2.—*Tests with Variety × Cayenne hybrids. Wilt following single infestations of 20 plants of each hybrid with 50 mealybugs per plant*

F₁ hybrids from crossing Cayenne with following varieties	Percentage of hybrid plants wilted when Cayenne wilted as follows:					
	10					
Red Spanish			31			
A. ananassoides	12	0		35		30
A. ananassoides 3N			30			
Ruby (Hybrid No. 8806)			40	45		...
Ruby*				10		
Queen (Hybrid No. 7789)					15	
do (Hybrid No. 7898)			5			
Pernambuco (Hybrid No. 8594)					20	
do (Hybrid No. 8597)				25		
do (Hybrid No. 9166)			20			
do (Hybrid No. 9243)						25
Monte Lirio (Hybrid No. 8728)		20	40			...
do (Hybrid No. 8752)		10	10			
do (Hybrid No. 8817)			0			
Natal (Hybrid No. 8971)						25

* Sarawak used instead of Cayenne as one parent of hybrid.

encountered. In addition, plantings made in plantation fields for agronomic comparison furnished additional evidence when accidental infestation of mealybugs occurred. It was in these plantings that the most striking evidence for resistance under conditions of plantation practice was obtained. The visual differences were accentuated by the dense growth habit of No. 7898 when surplus suckers were not removed and critical observation revealed many of the No. 7898 plants having the typical mild symptoms often present in this hybrid.

SYMPTOM EXPRESSION IN VARIETIES AND HYBRIDS

Factors influencing symptom expression are to be reckoned with in evaluating wilt incidence in hybrids and varieties other than Cayenne. Amount of anthocyanin materially affects the red and pink color reaction. In a broad-leaved succulent plant the downward drooping of the leaves characteristic of 3rd-stage wilt will be much more definite than in a stiff, narrow-leaved variety. In the narrow-leaved varieties, the inner reflexing of the leaf margins will be most pronounced; but so rigid and stiff is the leaf of some such varieties that symptoms rarely progress beyond the 2nd color stage, and downward bending of the leaves is limited to a dry distal portion. Varieties with pale green, almost-anthocyanin-free leaves, go through a sequence of yellow shades rather than red and pink as is the case with Cayenne. When differences in plant type and anthocyanin content are considered, however, there is normally no difficulty in diagnosis except in cases of extremely mild symptoms in varieties with which experience is limited.

Symptoms in hybrid No. 7898 are of special interest. This variety, as has been described, is low in anthocyanin and has a pea green color. It develops a lush wide-leaved growth as it approaches fruit maturity. The

suckers arising from the mother plant stem are numerous and tend to be narrow-leaved. Symptoms in this variety range from a slight yellowing of three or four adjacent median leaves to symptoms typical of Cayenne in every respect except for the development of the red and pink coloration and the progress to fourth stage in young plants, which occurs but rarely. There is a drying up of the fruit peduncle of this hybrid so that the fruit, as it matures to large size, cannot be supported and bends over (Fig. 1).

FIG. 1. Symptoms of wilt which occur in hybrid No. 7898 following several separate heavy infestations of mealybugs. Note the drooping fruit due to a drying peduncle.

DETAILED EXPERIMENTS WITH HYBRID NO. 7898

With the accumulated evidence from several years experience of the resistance of No. 7898 to wilt, about one acre was planted to this variety in the fall of 1941 for tests along the following lines: The effect of monthly infestations beginning in February, 1942, and continuing until July, 1942; single infestations to determine the sub-wilting effect, if any, of mealybug feeding; the effect on fruit and first ratoon suckers of late infestations made at monthly intervals from August, 1942, to October, 1942; the effect of removing excess ratoons on susceptibility to ratoon infestation; uncontrolled natural infestations.

Section 1. The effect of monthly infestations. Infestations were applied as indicated in table 3, beginning in February, 1942, and continuing until July, 1942.

TABLE 3.—*The monthly infestations of hybrid No. 7898 and Cayenne in 1942*

Variety and beds infested	Time of infestation	
No. 7898		
Beds 47–48	Feb., Mar., Apr., May, June	
Beds 49-50	Mar., Apr., May, June	
Beds 51–52	Apr., May, June	
Beds 45–46	May, June	
Beds 43–44	June, July	
Cayenne		
Bed 53	Apr., May, June	

It was clear, however, from detailed mealybug counts taken in August, that the successively applied mealybug colonies had not become permanently established and that the results, therefore, were due to the cumulative effects of the separate infestations rather than to the accumulation of huge mealybug populations.

Symptoms on these plants were recorded at intervals, and in table 4 the data up to December 29 are presented. It will be noted that fairly large percentages of No. 7898 are recorded as showing symptoms but the symptom expression was of a decidedly mild type. Figure 2 shows No. 7898 and Cayenne, each infested at three successive intervals. As a matter of fact, severe wilt was already manifest in many Cayenne plants at the time the third infestation was made and the collapse in the Cayenne was as complete and

FIG. 2. On the right is Smooth Cayenne with severe wilt, on the left is hybrid No. 7898. Both beds had received three separate infestations of mealybugs.

severe as has ever been experienced. The photograph, while not adequate to indicate the symptom on No. 7898, is sufficiently clear to indicate an extreme difference in the susceptibility of the two varieties. The symptom in No. 7898 can best be described as a slight yellowing of a few, usually three or four, adjacent median leaves; that in Cayenne as typical 4th-stage (severe) wilt (4). Some No. 7898 plants progressed to 3rd-stage wilt but even this was usually limited to the original median leaves, the center of the plant growing out apparently normally while this progression was occurring.

TABLE 4.—*Wilt records from plants infested with mealybugs from 2 to 5 times at monthly intervals. Beds 43–52, No. 7898. Bed 53, Cayenne*

Bed No.	Plot No.	No. of infestations	No. of plants in plots	Plants showing symptoms	
				No.	Per cent
43	A	2	100	33	33
	B	2	99	48	48
	C	2	100	63	63
	D	2	114	72	63
44	A	2	100	60	60
	B	2	100	44	44
	C	2	100	42	42
	D	2	106	54	51
45	A	2	100	40	40
	B	2	100	36	36
	C	2	100	42	42
	D	2	96	53	55
46	A	2	100	56	56
	B	2	100	50	50
	C	2	100	57	57
	D	2	83	43	52
47	A	5	100	69	69
	B	5	100	72	72
	C	5	100	75	75
	D	5	74	58	78
48	A	5	100	62	62
	B	5	100	68	68
	C	5	100	60	60
	D	5	65	41	63
49	A	4	100	70	70
	B	4	99	47	47
	C	4	99	69	70
	D	4	56	34	61
50	A	4	100	62	62
	B	4	100	53	53
	C	4	100	52	52
	D	4	44	22	50
51	A	3	100	45	45
	B	3	100	49	49
	C	3	100	46	46
	D	3	35	13	37
52	A	3	98	29	30
	B	3	100	44	44
	C	3	100	45	45
	D	3	25	8	32
53		3	694	694	100

In February. 1943, an attempt was made to add additional data to those recorded in table 4. but there were few plants with absolutely no evidence of at least mild symptoms. These symptoms, however. did not prevent the No. 7898 plants from maturing a crop. In the more heavily infested beds, there was some collapse in green fruit stage and many fruits fell over as they ripened, because of the weak and dried-up fruit peduncle. These latter, of course, did not produce slips, and a few scattered spots developed in the field where the plants finally died without producing ratoon suckers.

Just prior to plant crop harvest these heavily infested beds were examined to make selections of the more resistant individuals. Out of about 3500 plants in this section of the field, 14 were selected on the basis of showing no symptoms at all and 80 on the basis of showing only a very weak symptom. It was of interest that the symptom-free selections were all early-fruiting plants.

Section 2. Single infestations to determine the sub-wilting effect, if any, of mealybug feeding. In this experiment. five beds planted to hybrid No. 7898 and two beds to Cayenne were divided into plots 25 feet long, each containing 50 plants. Alternate plots were infested, and each infested plot was separated from another by a check noninfested plot of the same size. Infestations were again by the fruit-rind method and were made in March, April, May, and June, each month's infestation being made in a strip across the seven beds.

The results of these infestations are in table 5. Symptoms in No. 7898 were of an extremely mild type, with recovery rapid and commercial fruit maturing. Symptoms in the two Cayenne beds were typical but not the extreme type seen in the 3 infestation series mentioned previously (Table 4). Fruit weights and slip and sucker production were recorded for the plots of No. 7898. The infested Cayenne plots, while showing the typical recovery, had not recovered to fruiting by the end of the normal harvest time, and very few plants produced commercial-sized fruits even later. The only comparisons possible therefore are between infested and check plots of No. 7898 for the four separate infestations, one month apart. These records, which were taken in great detail, are summarized in table 6, which gives the analysis for significance in fruit yield as well as slip and sucker production for the four separate infestations. The March infestation resulted in the greatest reduction in all three indexes, but all four infestations reduced fruit yield, and slip and sucker production, in spite of almost negligible visual symptoms. The enhanced effect of the March infestation could be accounted for in comparison with the effects of the other three infestations by its earliness, but the results for April, May, and June are not consistent in this respect, the May infestation being out of line in fruit and slip records. The most likely explanation is to be found in the fact that the mealybugs for each infestation were naturally collected at different times and places, and the differences between infestations shown in table 6 are probably more likely

due to differences in the mealybugs rather than to plant age at time of infestation.

Section 3. The effect on fruit and first ratoon suckers of late infestations made at monthly intervals. In this test, two beds of Cayenne and four of No. 7898, all contiguous, were infested with single infestations made at monthly intervals from August, 1942, to October, 1942, by means of the fruit-rind method. Wilt resulting up to June, 1943, when the plant-crop fruit harvest was approaching, is recorded in table 7. There was some movement to check plants, but this was minor except in one Cayenne section.

TABLE 5.—*Wilt records from plants infested with mealybugs once. Beds 36–40, No. 7898. Beds 41–42, Cayenne*

Bed No.	Plot No.	Date of infestation	No. of plants	Plants showing symptoms	
				No.	Per cent
36	1	March	50	14	28
	2	April	50	9	18
	3	May	50	6	12
	4	June	50	16	32
37	1	March	50	12	24
	2	April	50	10	20
	3	May	50	6	12
	4	June	50	10	20
38	1	March	50	17	34
	2	April	50	12	24
	3	May	50	4	8
	4	June	50	14	28
39	1	March	50	17	34
	2	April	50	15	30
	3	May	50	6	12
	4	June	50	23	46
40	1	March	50	21	42
	2	April	50	18	36
	3	May	50	8	16
	4	June	50	15	30
41	1	March	49	43	88
	2	April	50	47	94
	3	May	50	45	90
	4	June	49	39	80
42	1	March	50	45	90
	2	April	50	45	90
	3	May	50	44	88
	4	June	50	28	56

The incidence of plants with symptom expression was not decreased as much with age of plant in No. 7898 as it was in Cayenne. (Compare tables 5 and 7.) This characteristic of Cayenne is well established from previous data (1, 4, 5). Wilt in the green-fruit stage of the plant's growth occurred in No. 7898 to the extent that 3.6 per cent of the total number of plants were recorded as showing symptoms.

Section 4. The effect of removing excess ratoons on susceptibility to ratoon infestation. In this test, following plant crop harvest of the plants

in Section 3, the surplus suckers were removed from the plants in alternate beds so that no more than two suckers were left on those plants. This particularly concerned No. 7898, which normally produces a dense mass of suckers which was thought might affect the susceptibility of the plants to infestations occurring during the ratoon growth. These infestations were made in December, 1943, and the first evidence of wilt was seen in March, 1944, with typical collapse of the ratoon Cayenne and with typical yellow areas on the distal half of the median leaves of No. 7898. By August, wilt had developed to an extreme degree.

TABLE 6.—*Effect on fruit weight and slip and sucker production of single mealy-bug infestations on infested No. 7898 plants which showed either no symptoms or only those of the mildest type, compared with healthy check plants. Analysis for significance between infested and check plots by "Student's" paired-plot technique*

Index	Comparison of plots infested vs. check	Mean difference	Standard error of the difference	t value[a]
Fruit (g.)	Infested March	409.4	48.96	8.362
	Infested April	273.2	72.52	3.766
	Infested May	150.6	40.38	3.730
	Infested June	283.2	64.80	4.370
Slip (No.)	Infested March	1.738	0.1533	11.337
	Infested April	0.638	0.2375	2.686
	Infested May	0.428	0.0739	5.792
	Infested June	0.720	0.1293	5.568
Sucker (No.)	Infested March	1.218	0.2028	6.006
	Infested April	0.726	0.2240	3.241
	Infested May	0.524	0.1568	3.342
	Infested June	0.504	0.1335	3.775

[a] The t value required for significance for odds of $19:1 = 2.776$; for odds of $99:1 = 4.604$.

In Cayenne, wilt was more extreme in the desuckered bed both with respect to number of plants involved and severity of symptoms. In No. 7898, symptom development was typical of that hybrid, with yellowed leaves having wilted tips, but there were scattered cases in which the whole sucker was involved as in Cayenne. Wilt was less extreme in the desuckered beds than in those in which the whole mass of suckers was left on. The increased drain of the larger number of suckers on the mother plant stump could have been the decisive factor in causing more extreme wilting among these plants.

Another curious result from this experiment was the effect the infestation in the fall of 1942 had on wilt resulting from the December, 1943, infestations. One-half of each plot in this test had been used for tests in Section 3 and had been infested in August, 1942, when the plants were about 10 months old. The other half of each plot had been a check in the previous test and had not been infested. Both halves received equal infestations in the sucker stage, but wilt following these infestations was clearly more severe in the sections which had been infested once before while the plants were young. Figure 3 illustrates this for No. 7898, but it was equally true for Cayenne.

The explanation for this phenomenon is obscure but a tenable hypothesis is that mealybug feeding affects tissues in such a manner as to injure without killing; later infestations add to the injury already sustained, with more severe wilt as a consequence of the cumulative effects.

The difference between symptom expression in the plots infested once (Table 5) and those infested several times at monthly intervals (Table 4) might also be amenable to the same explanation.

TABLE 7.—*Effect of single infestations made prior to blossom differentiation, August, 1942–October, 1942. Beds 25–26, Cayenne. Beds 27–30, No. 7898*

Bed No.	Section No.	Date of infestation	No. of plants	No. of plants showing symptoms
25	A	August	100	15
	B	Check —noninfested	100	3
	C	September	100	19
	D	Check—noninfested	99	0
	E	October	100	11
26	A	August	100	25
	B	Check—noninfested	100	9
	C	September	100	14
	D	Check—noninfested	100	10
	E	October	100	43
27	A	August	100	20
	B	Check—noninfested	100	0
	C	September	102	19
	D	Check—noninfested	100	0
	E	October	100	2
28	A	August	100	25
	B	Check —noninfested	100	0
	C	September	101	22
	D	Check—noninfested	100	0
	E	October	100	7
29	A	August	99	40
	B	Check—noninfested	100	2
	C	September	100	27
	D	Check—noninfested	100	4
	E	October	99	9
30	A	August	102	32
	B	Check—noninfested	100	4
	C	September	100	31
	D	Check—noninfested	100	4
	E	October	100	18

Section 5. Uncontrolled natural infestations. This is perhaps the most severe type of test to which plants can be subjected if the area in which they are growing is infested early in the plant's life and continues with only the normal fluctuations of an unsprayed infestation. Under these circumstances, no hybrid or variety has successfully maintained itself, although many have a considerable degree of resistance and do not have any large percentage of wilted plants until the ratoon stage.   Hybrid No. 7898 has maintained itself remarkably well up to plant crop with this type of infestation, but it has a very considerable degree of wilt with severe symptoms in ratoon.
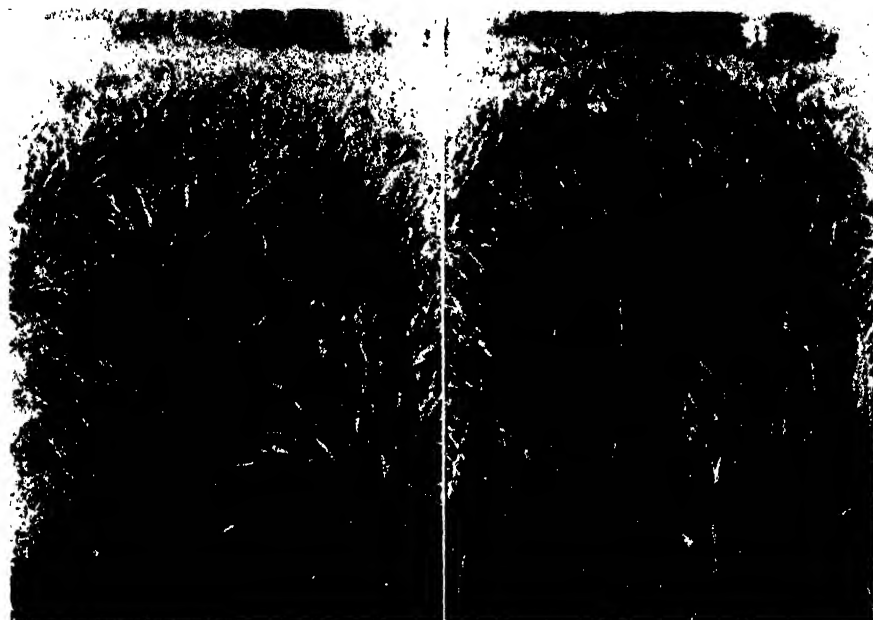
Fig. 3. *Left:* Hybrid No. 7898 susceptible to wilt following two mealybug infestations, the first prior to blossom differentiation of the first crop and the second at the ratoon sucker stage. *Right:* The same hybrid with a low incidence of wilt following a single mealybug infestation at the ratoon sucker stage.

In table 8 are the records of a number of varieties and hybrids which were selected for this test on the basis of their previous performance in resistance tests when a single infestation was used. They were planted in a small

TABLE 8.—*Wilt resulting from uncontrolled natural infestations, extending through both plant crop and ratoon, a period of about two years*

Variety or hybrid	No. of plants	Percentage wilted	
		Plant crop	Ratoon
Red Spanish	10	0	40
Cayenne	1723	15	97
Congo	25	0	92
Natal	13	0	69
Pernambuco	6	0	17
Queen	20	5	100
Ruby	27	4	100
Sarawak	21	0	95
Cayenne × Queen (No. 7889)	19	0	29
do (No. 7898)	16	6	69
do (No. 8050)	10	0	20
Cayenne × Monte Lirio (No. 8473)	15	40	100
do (No. 8705)	16	6	93
do (No. 8877)	15	40	100
Monte Lirio × Cayenne (No. 8728)	15	7	100
do (No. 8752)	25	12	88
do (No. 8817)	16	0	100
Pernambuco × Cayenne (No. 8597)	15	0	57

area bounded on two sides by wild vegetation, and on the other two sides by growing pineapples. Infestation did not occur very rapidly, so after the plants were well started, a few infested fruits were scattered throughout the plot. The table presents the data for wilt in both plant crop and ratoon, and out of a very long series of varieties and hybrids included in this test, representatives have been chosen to show three types of reaction, one where a continued infestation has resulted in a high percentage of wilt in plant crop, another where a low percentage of wilt in plant crop has been followed by severe wilt in ratoon, and a third which includes all the cases in which wilt in ratoon fell below 40 per cent. The low percentage of wilt in Cayenne in plant crop undoubtedly reflects the late start that the infestation got in this plot because the percentage of wilt in that group (15 per cent) in plant crop is lower than would normally be expected. It should be noted that although several of the hybrids showed no wilt up to inflorescence stage, none of these survived the continued infestation in ratoon, although there was some difference in the severity of the symptoms which developed.

SYMPTOM EXPRESSION IN NO. 7898 WHEN INFESTED WITH MEALYBUGS FROM THAT HYBRID

The toxin hypothesis of mealybug wilt has as a natural corollary the expectation that the nutrition of the mealybug on the plant from which it is moved might affect its toxicity to the plant to which it is moved. The significance of this in breeding programs for resistance is very great. Tests have naturally been conducted with bugs reared on the commercial variety, Cayenne, but in the event of a new hybrid being substituted for Cayenne, mealybugs would then move, under field conditions, within plantings of that new hybrid.

The first evidence that a variety could react differently to mealybugs which had been reared on that variety than to mealybugs from a different variety was available several years ago in a small test with Cayenne and Queen (the two parents of hybrid No. 7898). The first transfer using Cayenne bugs resulted in 60 per cent wilt in the Cayenne to Cayenne sequence and 10 per cent in the Cayenne to Queen sequence. When the mealybugs were transferred from these plants to a second series of test plants some months later, the Cayenne to Cayenne sequence gave 86 per cent wilt and the Queen to Queen 68 per cent.

In the large planting of No. 7898 in which most of the experiments with that hybrid thus far recorded were conducted, a small section at one end of the planting was left unsprayed. This became infested in due course but there were few plants showing any sort of symptom by plant crop, and the ratoon growth was vigorous. A new planting of No. 7898 was made next to this infested ratoon with the expectation that it would become infested with bugs from the latter. This occurred and the resulting effect on the young plants was significant. The symptoms resulting from this "hybrid to hybrid" infestation were typical of wilt in Cayenne. The entire plant

was involved, color changes were not limited to a few leaves, and there was considerable downward drooping of the plant typical of 3rd-stage wilt (Fig. 4). Many of these plants, however, recovered by fruiting time, but in some cases dried peduncles resulted in drooping fruit.

A field experiment was then devised to determine the effect of the variety to variety sequence of mealybug feeding. In this test, mealybugs from plantation Cayenne and from No. 7898 were used for cross infestations by the fruit rind method. These infestations were sprayed out after 26 days.
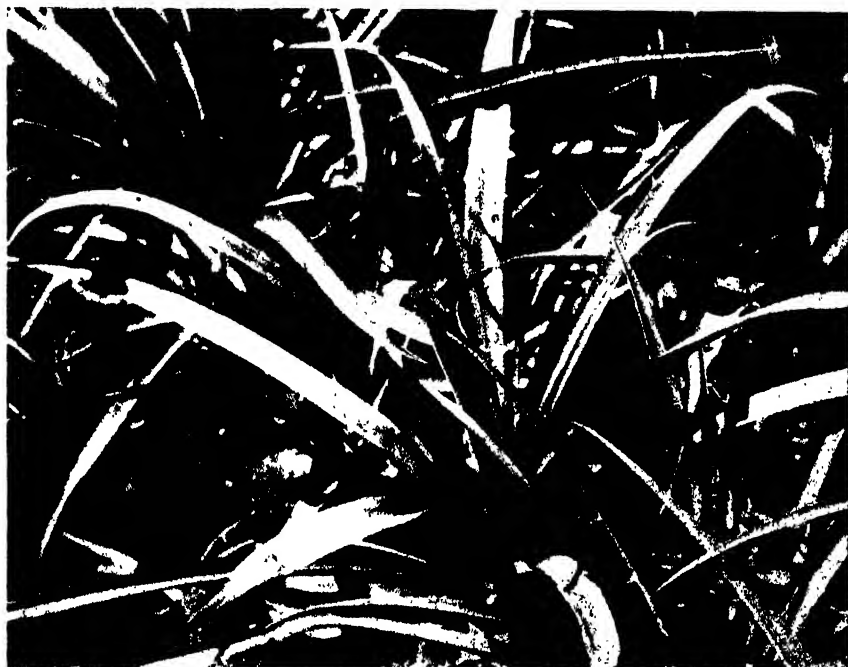
FIG. 4. Hybrid No. 7898 plant going into typical 3rd-stage wilt, as a result of natural infestation of mealybugs from an older planting of the same hybrid. The plant had a lighter color than nearby plants, the dry leaf tip, and the downward drooping of the median leaves.

The results of this test were significant in two respects. Symptoms on No. 7898 which were infested with mealybugs from No. 7898 developed 3rd-stage wilt typical of Cayenne. This confirmed the results in the field plot mentioned above. The effect of No. 7898 bugs on Cayenne, however, was unexpected. Wilt following this infestation had a shorter period for development of symptoms which progressed rapidly with a complete collapse of all the plants resulting. Mealybugs from Cayenne to Cayenne also wilted all the plants in that plot but development of symptoms was slow and plant collapse was not complete (Fig. 5).

This increase in toxicity to a susceptible variety, of mealybugs which are grown on a resistant variety, is probably unique. The nearest analogous phenomenon is perhaps the increased virulence to a susceptible corn variety

of *Phytomonas stewarti* grown in resistant varieties (6, 7), but the explanation on the basis of the segregation of genetic strains of the bacteria does not hold in the case of mealybugs because the change in toxicity has occurred in mealybugs following a single transfer to the resistant variety.[4]

TESTS OF HYBRID POPULATIONS HAVING THE RESISTANT HYBRID NO. 7898 AS ONE PARENT

Following evidence of resistance in the hybrid No. 7898 and in the varieties Queen and Pernambuco, individual plants of a hybrid population, Lot 1760, produced by crossing No. 7898 with No. 8597, the latter an F_1 Cayenne × Pernambuco selected clone, were planted as clones in the fall of 1941. These clones, with an average of 3.6 plants each, were infested with mealybugs by the fruit rind method early in 1942.
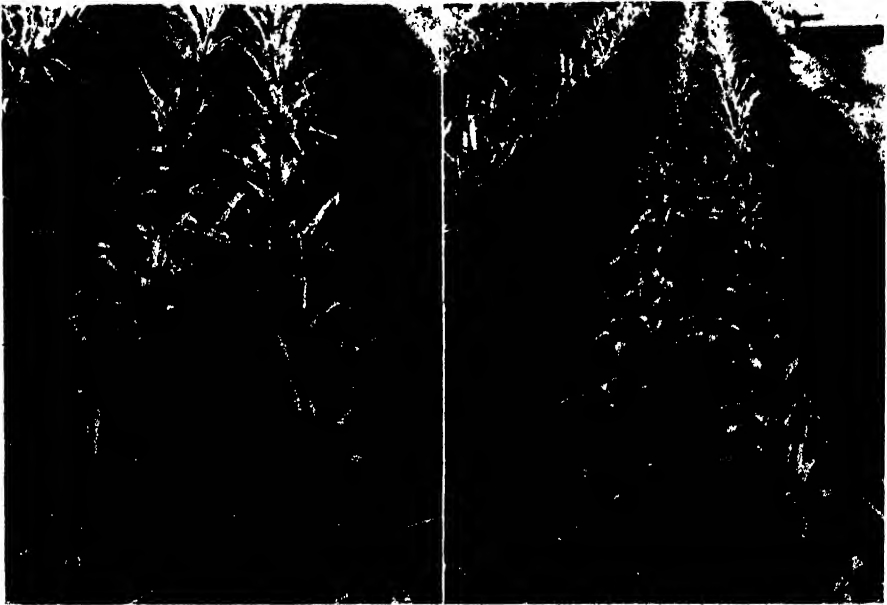
Fig. 5. Cayenne plants (right) infested with mealybugs from the resistant hybrid No. 7898; the same variety (left) infested with mealybugs from Smooth Cayenne.

Records of wilt occurring in these clones were made in August, 1942, when the plants were about 10 months old and again in July, 1943, at the age of about 21 months. Pineapple plants usually produce a mature fruit in 18 to 20 months and most of these hybrids had fruited or were in fruit at the time of the second wilt record in July, 1943.

Table 9 shows segregation of these clones into wilting and nonwilting groups, with susceptibility to wilt again appearing as dominant. The figures of 232 wilting to 65 nonwilting clones approximates a monogenic F_2 ratio. The phenomenon of recovery from wilt mentioned as characteristic of

[4] Unpublished data.

the No. 7898 hybrid is also shown in some of the clones of Lot 1760 derived from No. 7898 as one parent.   Table 9 lists 104 such clones which showed wilt following infestation with mealybugs when the plants were 10 months old but which were listed as symptom-free by fruiting time in 1943.

TABLE 9.—*Record of wilting in clones grown from single F$_1$ plants produced by crossing hybrid No. 7898 with No. 8597 (Lot 1760)*

Total number of clones	297
Number showing no wilt in 1942	91
Number showing no wilt in 1943	169
Number showing no wilt in both years	65
Number wilted in 1942 and recovered in 1943	104

The clones of Lot 1760 which were susceptible to wilt were discarded at the end of the 1943 test.   Clones appearing to be resistant were replanted in the fall of 1943 and again infested with mealybugs early in 1944 with the following records obtained in July, 1945: 69 clones wilting, 7 clones showing delayed wilt, 10 clones showing no wilt.

The tabular data presented here from numerous infested varieties and hybrids demonstrate that very rarely do all the plants of an infested susceptible group have wilt symptoms.   Because of this characteristic some of the small clones may fail to show wilt in one test but will do so in another.   However, the larger the clone the more readily are wilt symptoms detected in that clone if it is a susceptible one.

Throughout these experiments clones were classified as susceptible when they contained a single wilting plant.   As was pointed out, the first clonal generation of Lot 1760 hybrids contained an average of 3.6 plants per clone.   Some of these classified as resistant in the 1942 and 1943 records showed wilt in the second vegetative generation test in 1945.   The number of plants per clone in the second generation (14.6 plants per clone) was large enough to give increased confidence in the segregation of resistant from susceptible clones.

Both the parents of Lot 1760 were derived from varieties (Queen and Pernambuco) which were more resistant to wilt than the Cayenne variety. A number of the clones discarded after the 1943 tests were similar to the No. 7898 parent in degree of resistance and ability to recover from wilt. Selections, however, were directed toward obtaining a higher degree of resistance than was found in the parental varieties.

SUMMARY

Pineapple varieties collected from widely separated geographical areas have been tested for resistance to mealybug wilt.   From these tests it was determined that Cayenne, the commercial variety in Hawaii, is one of the most susceptible.

Tests with variety hybrids, particularly those in which Cayenne was used as one of the parents, indicate that susceptibility is a dominant character.

Detailed tests have been made with a Cayenne-Queen hybrid (No. 7898) for which resistance was first demonstrated, in comparison with Cayenne, in commercial plantings under conditions of natural infestation.

This hybrid shows only mild leaf symptoms under conditions of mealy-' bug infestation which will produce severe symptoms in Cayenne. Under more severe conditions of infestation, namely, repeated infestations at monthly intervals, and continuous infestation, symptoms are severe and approximate those seen in Cayenne. Mealybugs grown on No. 7898 induce more typical symptoms on No. 7898 than do mealybugs from Cayenne. Symptoms on Cayenne appear more rapidly and develop to much greater severity when mealybugs from No. 7898 are used.

The susceptibility of Cayenne diminishes markedly with age of plant but that of hybrid No. 7898 does not appear to be affected by this factor to the same degree. Infestations of both Cayenne and No. 7898 made just before the differentiation of the inflorescence, increase the susceptibility of the same plants to infestations made a year later on the ratoon suckers.

Sub-wilting effects in No. 7898 plants which have shown only a mild leaf symptom, express themselves in reduced fruit size and reduced number of vegetative slips and suckers.

Studies with hybrids in which No. 7898 is one of the parents show evidence of segregation of the resistant character.

PINEAPPLE RESEARCH INSTITUTE,
　　UNIVERSITY OF HAWAII.

LITERATURE CITED

1. CARTER, WALTER. The pineapple mealy bug, *Pseudococcus brevipes*, and wilt of pineapples. Phytopath. **23**: 207–242. 1933.
2. —————. Mealy-bug wilt and green spot in Jamaica and Central America. Phytopath. **24**: 424–426. 1934.
3. —————. The geographical distribution of mealybug wilt with notes on some other insect pests of pineapple. Jour. Econ. Ent. **35**: 10–15. 1942.
4. —————. Some etiological aspects of mealybug wilt. Phytopath. **35**: 305–315. 1945.
5. —————. The influence of plant nutrition on susceptibility of pineapple plants to mealybug wilt. Phytopath. **35**: 316–323. 1945.
6. McNEW, GEORGE L. Factors influencing attenuation of *Phytomonas stewarti* cultures. Jour. Bact. **39**: 171–186. 1940.
7. WELLHAUSEN, E. J. Effect of the genetic constitution of the host on the virulence of *Phytomonas stewarti*. Phytopath. **27**: 1070–1089. 1937.

NOTES ON A BROWN PIGMENT AND OTHER UNUSUAL CHARACTERS IN CULTURES OF BACTERIUM MARGINATUM[1]

L U C I A   M c C U L L O C H

Bacterium marginatum L.McC., when grown on beef media, has no particularly distinctive characters, either macroscopic or microscopic; but on Thaxter's potato agar[2] a brown pigment and several unusual forms are produced so consistently and readily that this medium becomes the best means of identification of the organism.

The unusual color and forms characteristic of this important gladiolus pathogen are here briefly described with the hope that someone will be interested in carrying on biochemical investigations of the nature of the pigment and why it is deposited, almost entirely, in the bacteria and certain kinds of associated spheres. The "starch crystals" previously described[3] also deserve further investigation.

Detailed notes, photographs, drawings, microscope slides, and dry specimens are deposited in Mycological Collections, U. S. Bureau of Plant Industry, Soils, and Agricultural Engineering. Beltsville, Md.

Cultures of *Bacterium marginatum* may be obtained from the American Type Culture Collection, 3900 Reservoir Road, Washington 7, D. C.

Bacterium marginatum on Thaxter's potato agar[4] first produces a white, thick and soft growth later collapsing into a thin brownish layer. Cultures 1 to 4 days old have, along with the numerous bacteria, thousands of small hyaline spheres, mostly mere points in size; but many are 4 to 7 μ and others up to 20 μ in diameter. Material mounted for microscopic examination is opaque because of light refracted from the numerous spheres. A drop of 1 per cent NaOH placed at the edge of the cover glass causes the almost instantaneous disappearance of the spheres, leaving a transparent mount of bacteria. These spheres form as soon as growth is established and persist only as long as the medium is acid in reaction. During growth the medium, which is acid, increases in acidity, the pH reaching 3.8 to 4.0. As the peak of growth passes, the acidity declines until, with completed growth, the pH value is 8.0 to 8.6.

---

[1] McCulloch, Lucia. A bacterial disease of gladiolus corms and leaves. Jour. Agr. Res. [U.S.] 29: 159–177. 1924.

[2] Prepared according to formula given in Bitancourt, A. A., and Anna E. Jenkins. New discoveries of Myriangiales in the Americas. Proc. Eighth Amer. Scient. Congress, Washington, D. C., 1940. 3 (Biological Sciences): 154. 1942.

[3] McCulloch, Lucia. Starch-like radiate crystals produced by Bacterium marginatum in starch media. Jour. Agr. Res. [U.S.] 39: 495–501. 1929.

[4] All descriptions and illustrations are from Thaxter's potato-agar cultures unless otherwise noted.

These hyaline spheres stain readily, but seldom take the same color as the bacteria. In osmic acid they become black and brittle. Boiling distorts them slightly or produces a granular appearance. Dried on a glass slide and held in a blue flame they show no visible change in 20 seconds, but with 30 seconds of flaming they disappear. Pressure on the cover glass flattens some of the larger spheres and continued pressure causes dissolution and disappearance.

As the hyaline spheres disappear, a brown color begins to develop in the growth and reaches its maximum in 7 to 10 days. This brown color varies from pale amber to "tawny russet" and "raw umber" of Ridgway.[5] Examination shows that the color is not uniformly diffused but occurs in definite areas, usually as a fine network of branching lines (Fig. 1, E). Microscopic examination shows that the brown areas are composed of closely packed bacteria and spheres, translucent, golden brown to opaque dark brown, with an inconspicuous number of normal hyaline bacteria. Some of the brown bacteria are normal in size and shape but many are abnormal (Fig. 1, B).

Because of their number and size the brown spheres (Fig. 1, A) are more noticeable than the brown bacteria. Spheres 0.5 to 0.8 μ predominate, those 5.0 to 25.0 μ are abundant, a few are 40.0 to 45.0 μ, and one of 60.0 μ was observed. With age, if the culture medium remains sufficiently moist, the brown spheres, particularly the larger ones, develop a hyaline wall (Fig. 1, C, a). Soaking in cold or hot water usually causes the wall to appear. Pressure and movement of the cover glass flattens some of the larger spheres temporarily, and occasionally a sphere breaks into irregular fragments or into several smaller spheres. The wall, if present, usually breaks and separates from the brown center (Fig. 1, D). In moist Van Tieghem cells, kept under observation for long periods, some spheres developed the hyaline wall and also showed a further development in the breaking up of the wall into a number of small, hyaline spheres which eventually disappeared (Fig. 1, C, b–g).

The origin of the brown spheres is unknown. Possibly they may be the results of deposition of pigment in a dead bacterial cell which may then enlarge to form a sphere, and a capsule which is not pigmented swells to form the hyaline wall. (*Bacterium marginatum* has very definite capsules.) Secondly a granule from a bacterial cell may become pigmented and swell to form a sphere, but without a hyaline wall.

This theory would account for the fact that there seem to be two types of spheres, the walled or capsulated spheres, usually large, translucent, and apparently mere empty shells, and spheres lacking walls, dark or even opaque, and, when broken, divided into smaller but still opaque parts.

The brown spheres and bacteria persist indefinitely, even in old, dry cultures. They are extremely resistant to dry heat, to steam (15 lb. pres-

[5] Ridgway, Robert. Color standards and nomenclature. 43 pp. 53 color plates. Washington, D. C. 1912.
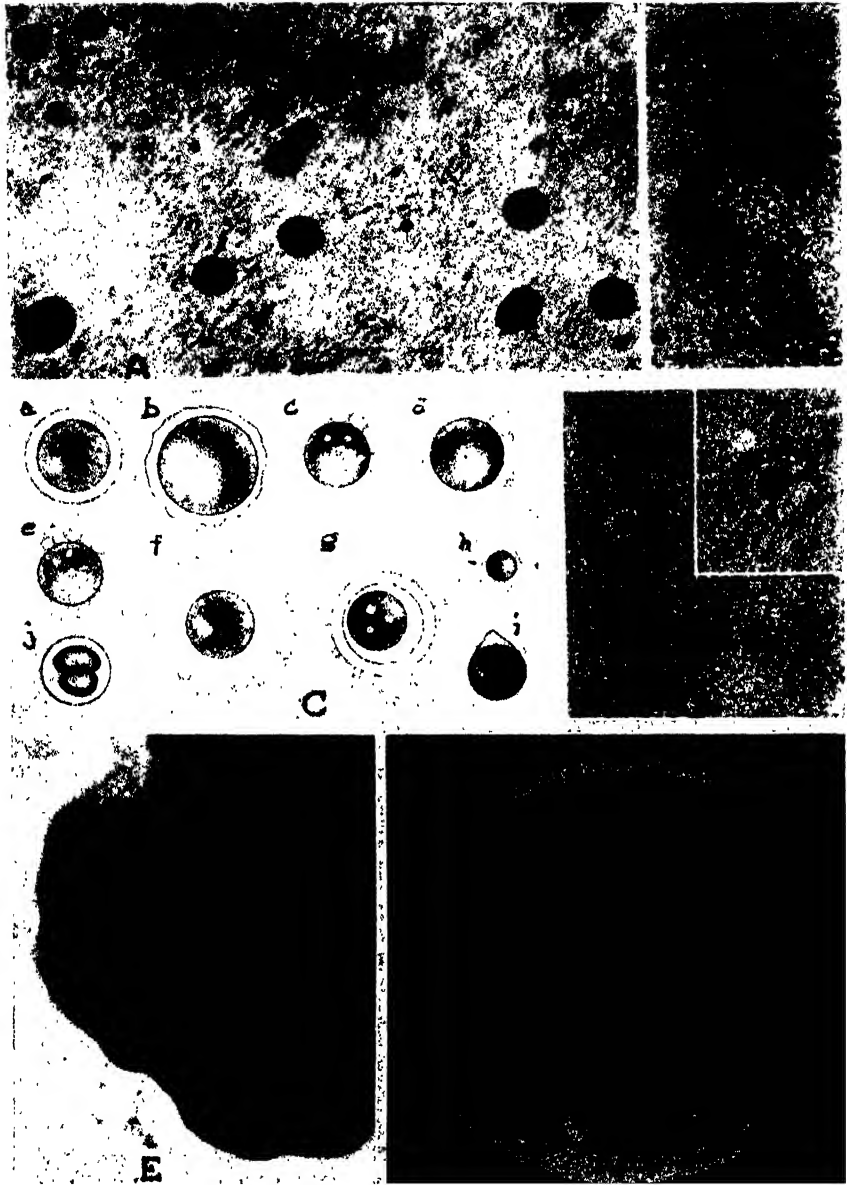
FIG. 1. A. Brown spheres. Not stained. × 600. B. Brown bacteria and three small brown spheres. Not stained. × 750 approx. C. Brown spheres with hyaline walls in various stages of dissolution. Not stained. (Drawings by L. C. C. Krieger from camera lucida drawings and measurements from Van Tieghem cell cultures by L. McC. The measurements given are the total diameters of the sphere and its wall.) a. Hyaline wall, uniform in thickness. Common type in cultures. 14 μ. b. Wall irregularly thick. Early stage of breaking up. 18 μ. c and d. Later stages in wall disintegration. 14 μ and 14.8 μ. e. Same sphere as c but 11 days later. 14 μ. f. Same sphere as a but 9 days later. 23 μ. g. Double wall and projections. 23 μ. h. Several attached hyaline spheres. 29 μ. i. Example of irregular wall. 9 μ. j. Two spheres in one wall. 11 μ. D. Brown spheres, wall of one broken by pressure. × 400. E. Section of a colony showing the brown lines. × 6. F. Plate culture showing clearing of the agar. × ⅓.

sure), to boiling water, and to alcohols; and they are attacked only slightly, if at all, by acids and alkalies. The only means so far discovered for destruction of these brown bodies, is subjecting them to a blue flame for 2 to 3 minutes.

Slight bleaching was caused by concentrated glacial acetic acid, concentrated carbolic acid, 5 to 50 per cent HCl, 4 to 25 per cent NaOH, aqua regia, and strong ammonia.

Slight darkening was caused by concentrated HCl, 50 per cent NaOH, and 50 per cent H_2SO_4.

Extreme darkening, even to blackening, was caused by concentrated H_2SO_4, 4 per cent osmic acid, and a saturated solution of picric acid.

These changes usually required exposure to the reagents for several days and sometimes for weeks. Acids in which brown bodies have been soaked become more or less brown even when there is no visible loss of color in the spheres. The addition of water to the browned acid produces a brown precipitate.

The originally rather cloudy potato agar becomes more nearly transparent as the bacterial growth develops. In plate cultures the clearing begins at the margin of the colony and spreads until all of the agar is cleared (Fig. 1, F). In slanted agar tubes the clearing begins at the top of the slant and, if the agar is deeply slanted, continues to the base of the tubes. The cleared agar is negative for reducing sugars and the starch is apparently not lessened.

Imbedded in the cleared agar, sphero-crystals composed of radiating, needle-like parts are abundant. These are 15 to 35 μ in diameter, a few being somewhat larger. These crystals are formed only in media containing starch and the fact that they become blue when treated with iodine solutions suggests that they may be some form of starch. They are referred to by the writer as "starch crystals."

Mycelium-like forms (Fig. 2, A, B, C) develop occasionally in the potato agar and abundantly in 2 to 3 per cent solutions of dextrose or galactose. These forms, 2 to 200 μ long, are mostly slender with smooth continuous walls while others are wholly or partly like chains of bacteria. They have considerable motility in young cultures. Most of these filamentous forms are hyaline but in agar cultures a few are yellow to brown.

For the production of hyaline spheres, of brown spheres and bacteria, and "starch crystals," Thaxter's medium seems to be the most generally favorable and reliable. Best results were obtained with pH values of 5.0 to 5.5. Other media were tried in an effort to determine which substances are necessary for the production of the different forms. The experiments are far from comprehensive but they do demonstrate that neither potato nor starch are necessary for the development of the brown forms. Typical brown bacteria and brown spheres were produced in synthetic media. Starch is essential for the development of "starch crystals." The myelium-like forms develop best in 2 to 3 per cent solutions of dextrose or galactose.

Brown forms have been found, but not abundantly, in the pellicles of the dextrose-solution cultures.

Numerous other species of bacteria causing plant diseases were grown parallel with *Bacterium marginatum*, but none of these ever showed a trace of the pigment of forms so common in *Bact. marginatum*.
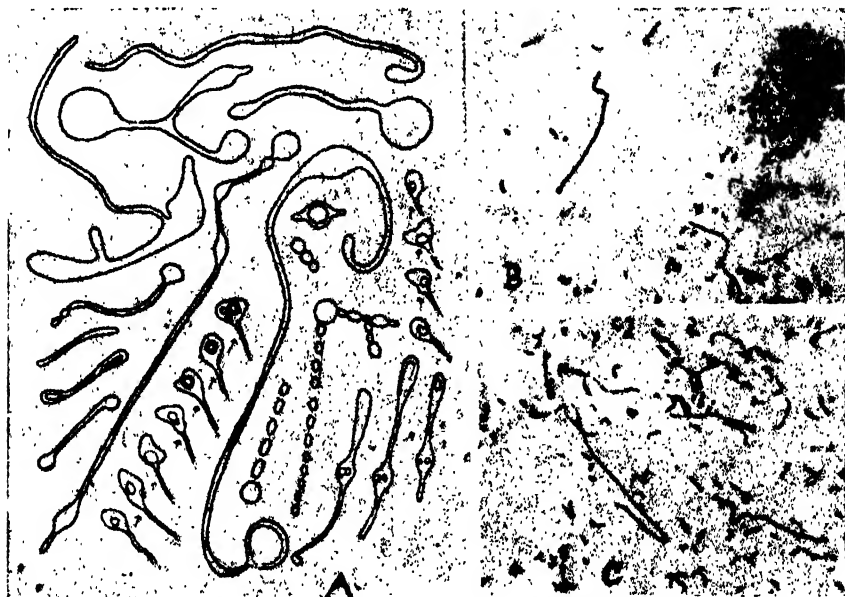
Fig. 2.   A. Mycelium-like forms of *Bacterium marginatum*.   Camera lucida sketches. Approx. 800×.   B, C. Photographs of the mycelium-like forms, stained and much shrunken.

SUMMARY

Bacterium marginatum L. McC., when grown on suitable media, produces in addition to the usual bacterial cells great numbers of small, evanescent, hyaline spheres.   These soon disappear and are followed by less numerous but larger brown spheres which are permanent and extremely (practically entirely) resistant to the many chemical reagents tried.   Brown bacteria also occur.   The brown pigment is almost entirely confined to the bacteria and the spheres.

In the cleared parts of the agar cultures, below the bacterial growth, sphero-crystals 5 to 35 μ are abundantly produced.   Since these crystals are formed only in media containing starch and become blue when treated with iodine solutions, it is thought they may be some form of starch.

In solutions of dextrose or galactose (2 to 3 per cent) numerous, motile myceliumlike forms develop.

BUREAU OF PLANT INDUSTRY, SOILS, AND
AGRICULTURAL ENGINEERING,
    U. S. DEPARTMENT OF AGRICULTURE,
        BELTSVILLE, MARYLAND.

TEST TUBE DILUTION TECHNIQUE FOR USE WITH THE SLIDE–GERMINATION METHOD OF EVALUATING PROTECTANT FUNGICIDES[1]

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, COMMITTEE ON STANDARDIZATION OF FUNGICIDAL TESTS

The test tube dilution technique is eminently suited for preliminary slide-germination tests of new chemicals as fungicides. It is more simple than the horizontal sprayer or settling tower technique, is more rapid than the latter and no elaborate apparatus is required. For water-soluble materials it is the most precise technique, while for chemicals giving ready and stable suspensions it is reasonably accurate. It is not accurate for difficultly suspendible chemicals and cannot be used for laboratory "rain" tests of tenacity. The technique as described here is an elaboration of one previously developed (3, 4). It should be used in conjunction with the standard slide-germination method (1) in which there is given the necessary background information and relevant detail of supporting techniques.

APPARATUS

Test tubes without lips, 150 × 18 mm. (1 for each dilution of each chemical and each fungus). Transfer pipettes—5 ml. (1 for each chemical)—2 ml. (1 for each chemical and each fungus). Two-ml. pipettes graduated in 0.5-ml. units (1 for each fungus). Test tube wire support racks to hold 40 tubes each (at least 1 for every 10 chemicals and every fungus). Graduate cylinders—50 ml. (1 per chemical). Erlenmeyer flasks—250 ml. (1 per chemical). Other general apparatus as ordinarily required for the slide-germination method.

PREPARATION OF DILUTION

Unless otherwise stated, rapid and approximate dilution methods are used, since the errors thus introduced ordinarily do not equal or exceed day to day variation (3, 4). Weigh out 0.5 gram of the chemical to be tested, dissolve or suspend in 50 ml. of distilled water, measured from a 50-cc. graduate cylinder and placed in a 250-ml. Erlenmeyer flask. After thorough shaking, withdraw 2 ml. by means of the 2-ml. transfer pipette and place in a test tube, being careful not to run down inner sides of tubes. Place test tube in wire support rack and label tube with wax pencil to identify compound. Repeat for each fungus using a different rack.

Dose Ratio of 10. Withdraw 5 ml. from the stock one per cent (10,000 p.p.m.) preparation by means of a 5-ml. pipette, discard remainder from flask, rinse rapidly from wash bottle, and add the 5-ml. sample. Add 45 ml. of distilled water, shake thoroughly. Rinse the 2-ml. pipette in this new dilution, discard rinsing solution then withdraw 2-ml. samples as before, and place in test tubes which in turn are placed beside the first tube in the rack. In case of 4 dilutions or less, run tubes crosswise of rack, if more than 4, place lengthwise. No label is needed as the compound and dilution are identified by position. Continue to dilute as above until a series of 4 or 5 test-tube dilutions are obtained, giving 10,000, 1000, 100, 10, and 1 p.p.m. of chemical. The first or last in this series frequently may be omitted, depending on circumstances.

Graduate Cylinder Alternate. After first concentration is made and the necessary 2-ml. samples placed in test tubes, add 5 ml. to a 50-ml. graduate cylinder and add distilled water to make 50 ml. Agitate thoroughly by pouring back and forth into the 250-ml. Erlenmeyer flask or beaker. Withdraw necessary 2-ml. samples from flask for

the test tubes. Add 5 ml. to the graduate cylinder which has been rinsed, and make up to 50 ml. with distilled water. Continue dilutions in same manner until all desired are obtained.   The 2-ml. pipette is kept in the test tube for later use.

The dilution series for the second chemical is prepared as for the first, except, of course, fresh glassware is used throughout. The first test tube containing the most concentrated chemical is likewise labeled and placed in the wire support rack beside the corresponding one for the first chemical. Additional dilutions and tubes are placed side by side with the first series, so that in one axis of the wire support rack there is a series of the same chemical and in the other a series of the same dilution.

Dose Ratio of $\sqrt{10}$. This series usually follows preliminary results obtained with a dose ratio of 10. Start with lowest dilution giving no germination. For second dilution, place in 50-ml. graduate 16 ml. of first dilution, and 34 ml. of distilled water. Agitate thoroughly by pouring into 250-ml. Erlenmeyer flask as above and withdraw necessary 2-ml. samples for test tubes. Add 16 ml. from flask to graduate, and 34 ml. of distilled water, agitate as before, withdraw 2-ml. samples as before. Continue dilution series as far as desired.

More Accurate Dilution. In case of tests with soluble materials where high precision is desired or a very low dosage is used, it will be necessary to employ more orthodox methods of dilution. Here volumetric flasks in conjunction with pipettes or burettes should be used and the whole series prepared and placed in a series of Erlenmeyer flasks before the 2-ml. aliquots are withdrawn.

SPORE GERMINATION STIMULANT

In many cases it is necessary to add a spore stimulant to insure a high and relatively stable percentage of germination in the checks. For this purpose various agents have been employed, *e.g.*, orange juice (4), potassium citrate plus sucrose (5), extracts from dried potato-dextrose agar (6), and coenzyme R (2). The stimulant used should be stated.

Orange Juice. Orange juice stimulant is prepared by filtering the juice of several good quality oranges through cheesecloth, followed by filter paper, and finally through a large Berkefeld cylinder, type W, under vacuum. The resulting clear filtrate is diluted ten-fold with distilled water and 10-ml. portions placed in small corked shell vials which are stored at below freezing temperatures. This constitutes 10 per cent ultra filtered orange juice. When needed for use, dilute contents of one vial to 100 ml. which gives the desired concentration to add to the spore suspension (see below); the final concentration in which the spores are exposed for germination will be 0.1 per cent. Enough stock orange juice may be prepared and frozen to last several years. New batches may be expected to differ slightly in potency but may be standardized against the old if desired.

Other Stimulants. Details regarding the use of other stimulants may be seen in the original papers cited above.

ADDING SPORES AND STIMULANT

Spores of the desired fungus are suspended in water according to the standard method (1). A concentration of 500,000 spores per ml. should be attained by means of a counting cell. To an equal volume of the spore suspension there is added an equal volume of the spore stimulant solution of a concentration 10 times that finally desired. If no stimulant is added the initial spore suspension concentration should be reduced to 250,000 per ml. The mixture is well stirred by blowing through a 2-ml. pipette graduated in 0.5 ml. units; 0.5-ml. samples of the spore suspension stimulant mixture are then pipetted into each test tube containing the 2 ml. of diluted chemical. Before withdrawing each pipetteful the mixture must be thoroughly stirred by blowing. One pipetteful will suffice for the 4 test tubes arranged crosswise of the support rack, and the place between rows of "spores added" and "not added" may be simply indicated by moving each tube in turn one space sidewise, or by changing the slope of the tubes or by laying a pencil or pipette between the rows.

PLACING DROPS ON SLIDES

Four or five glass slides are placed in each moist chamber in a horizontal position to the operator and two drops from each test tube of spores and chemical are pipetted side by side on to the left-hand side of a glass slide. For this purpose the 2-ml. transfer pipettes employed to place the most dilute chemical in the test tube are again used. However, fresh pipettes will be needed for each extra set of fungi and chemicals. Hence when pipetting work from the most dilute chemical to the most concentrated and from the top or furthermost slide to the nearest, the second fungus is placed on the right-hand

side so that similar dilutions are placed on the same slide, and only one chemical is in a single moist chamber. If a given chemical is volatile and toxic and the range of dilutions is wide, the compound may be overrated because of the volatilization from concentrated drops and resulting condensation in dilute drops. This will be corrected in a second test where a lower dose ratio and smaller range of dilutions is used.

When the chambers are filled, the "tops" are placed in position, the water seal added and germination counts made 20-24 hours later, according to the standard method.

LITERATURE CITED

1. AMERICAN PHYTOPATHOLOGICAL SOCIETY, Committee on Standardization of Fungicidal Tests. The slide-germination method of evaluating protectant fungicides. Phytopath. **33**: 627–632. 1943.
2. GOLDSWORTHY, M. C., and E. L. GREEN. Effect of low concentrations of copper on germination and growth of conidia of *Sclerotinia fructicola* and *Glomerella cingulata*. Jour. Agr. Res. [U.S.] **56**: 489–505. 1938.
3. McCALLAN, S. E. A., R. H. WELLMAN, and FRANK WILCOXON. An analysis of factors causing variation in spore germination tests of fungicides. III. Slope of toxicity curves, replicate tests, and fungi. Contrib. Boyce Thompson Inst. **12**: 49–77. 1941.
4. McCALLAN, S. E. A., and FRANK WILCOXON. An analysis of factors causing variation in spore germination tests of fungicides. I. Method of obtaining spores. Contrib. Boyce Thompson Inst. **11**: 5–20. 1939.
5. MILLER, HAROLD J. The use of *Venturia inaequalis* and *Sclerotinia fructicola* with pure chemical stimulants in slide germination tests of fungicides. Phytopath. **44**: 1009. 1954.
6. PETERSON, P. D. The spore germination method of evaluating fungicides. Phytopath. **31**: 1108–1116. 1941.

STANDARDIZED SPRAY NOMENCLATURE[1]

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY
COMMITTEE ON STANDARDIZATION OF FUNGICIDAL TESTS

The standardized spray nomenclature for deciduous fruit trees, here presented, has been attained through the cooperation of plant pathologists, entomologists, and horticulturists representing the various pertinent regions of the United States. The terms employed are believed to express the best general and current usage.

DOSAGE

All spray materials are to be expressed in standard units of weight (avoirdupois) or liquid measure (U. S. System) sufficient to make 100 gallons of spray mixture. Examples:

"*Bordeaux mixture 8–8–100*," or 8 pounds of copper sulfate, 8 pounds of lime (hydrated or burnt) with sufficient water to make 100 gallons of spray mixture.

"*Liquid lime sulfur 2–100*," or 2 gallons of liquid lime sulfur (32° B = 1.2823 sp. gr.) in sufficient water to make 100 gallons of spray fluid.

"*Lead arsenate 3–100*," or 3 pounds of lead arsenate with sufficient water to make 100 gallons of spray mixture.

All dust materials to be expressed in standard units of weight (avoirdupois) sufficient to make up 100 pounds of dust. Examples:

20–80 copper-lime dust, or 20 pounds of copper sulfate mixed intimately with 80 pounds of hydrated lime.

20–80 basic copper arsenate sulfur dust, or 20 pounds of basic copper arsenate mixed intimately with 80 pounds of 300 mesh or fine elemental sulfur or any equivalent of a proprietary conditioned sulfur.

1 per cent Rotenone dust, or 1 pound Rotenone (extract or in crushed roots) mixed intimately with talc, bentonite, chalk, tobacco dust, walnut flour, wheat flour, as the case may be, to make 100 pounds of dust mixture.

PART 1—APPLE AND PEAR SPRAY TERMS

Dormant.—From time the leaves have fallen in the fall until the buds show silver tips in the spring is regarded as dormant spray period. Application should never be made when temperatures approach freezing, but preferably at temperatures about 40° F.

Pre-bloom.—From time blossom buds begin to show the silver tip stage until first blossoms open.

Delayed dormant.—When green tips begin to show until leaves of blossom buds are one-fourth to one-half inch exposed.

Pre-pink.—When leaves are separated, leaving individual flower buds exposed in a tight cluster. Often referred to as "early closed cluster," "early pre-pink," "early pre-bloom," "pre-bloom," "late closed cluster," or "beginning of closed cluster." Temperature dominates the passage of one stage to another and for spraying purposes all stages should be considered as "pre-pink" since it is rarely possible to time this spray to the separate stages.

Pink.—When the flower buds have separated in the cluster, exposing the pedicel and the petals of each flower bud but not the stamens and pistils. Often referred to as "open cluster," "pre-pink," or "full pink."

Bloom.—When the flower buds have opened, exposing the stamens and pistils in from 50 to 75 per cent of the flowers. Full bloom corresponds to having 90 per cent or more of flowers open.

Petal fall.—When at least 75 per cent of the petals have fallen and before the calyx lobes have closed, often referred to as "calyx."

Cover sprays.—When the calyx lobes have closed and the fertilized fruits begin to increase in size. The sequence of the different cover sprays would follow the particular conditions existing in the different fruit sections and the application should follow closely the recommendations of the local authorities or be closely aligned to the spray schedules outlined in each state. These local recommendations are usually arrived at after years of study and the industry is usually informed by telephone, radio, and by letter of any

last minute changes to fit the conditions of the season. The cover sprays are usually designated according to their sequence: first, second, third, etc., or first brood and second brood sprays when referring to infestation of codling moth.

PART 2—PEACH, PLUM, APRICOT, AND ALMOND SPRAY TERMS

Dormant.—From time the leaves have fallen in the fall until the buds begin to swell the following spring is regarded as the dormant spray period.

Early pink bud.—When buds have emerged or burst but petals are only partially exposed; known as "red bud" in apricot and "bud swell" in almond.

Pink bud.—When buds begin distinctly to show the full color of the petals. Known as "late red bud" in apricot, "popcorn" in almond, and "puff ball" in peach.

Bloom.—When 10 per cent to 75 per cent of the blossoms are fully open.

Petal fall.—When 75 per cent of the petals have fallen.

Cover sprays.—When the young and growing fruits have been completely separated from their enveloping calyces. First cover known as "shuck fall" spray for peach. Cover sprays usually not needed in apricot and almond.

PART 3—CHERRY SPRAY TERMS

Dormant.—From the time the leaves have fallen in the fall until the buds begin to swell the following spring is regarded as the dormant spray season.

Pre-bloom.—When most of the buds show color of petals prior to full opening.

Petal fall.—When 75 per cent of the petals have fallen.

Cover sprays.—When the "shucks" (calyces) have fallen completely from the fruits, usually a week to 10 days after petal fall and including a spray 10 days before harvest and one following harvest.

PART 4—QUINCE SPRAY TERMS

Dormant.—From the time the leaves have fallen until the buds swell the following spring is regarded as the dormant season.

Bloom.—When the flowers are 90 per cent or more in full bloom.

Cover sprays.—When the calyx lobes are closed and the young fruits are expanding.

REPORT OF THE TWENTY-EIGHTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 28th annual meeting of the Pacific Division of The American Phytopathological Society was held in Berkeley, California, June 24 through June 27, with the Division of Plant Pathology of the University of California as host. A record attendance was had with 70 members and 10 non-members present, representing Arizona, British Columbia, California, Nevada, Oregon, Utah, Wisconsin, and Washington. The program consisted of four sessions of scientific papers, two business meetings, one day of field trips, and a 3-session Fruit Tree Virus Symposium. At one of the business meetings it was voted that the Secretary-Treasurer serve as Councilor with the A.A.A.S., Pacific Division.

The following officers were elected for 1947:

> President: C. E. YARWOOD
> Vice President: L. C. COCHRAN
> Secretary-Treasurer: GEORGE W. FISCHER (2 years)
> Councilor: MAX W. GARDNER

Following are the abstracts submitted:

Perennial Nightshade, a Host for Corynebacterium michiganense. R. C. BAINES. Perennial nightshade plants (*Solanum douglasii*) affected with stem cankers were found in a tomato field in southern California in 1944. Cultures of a bacterium similar to *Corynebacterium michiganense* were isolated and produced typical infection on nightshade and tomato plants. An isolate from tomato was pathogenic on tomato but not on nightshade. *C. michiganense* was found to overwinter on the perennial nightshade.

Heterosporium Disease of Nasturtium and Its Control. KENNETH F. BAKER. The extensive nasturtium seed fields in coastal California often sustain severe damage in mid-summer from an undescribed Heterosporium leaf spot. This production disease is largely limited to California. The fungus, internally carried in seed up to 47 per cent, infects the stem from the old hypogeally germinated seed and after the plant breaks over at soil level, sporulates abundantly. Infection of the leaves occurs through hydathodes and insect injuries, and, by harvest, plants often are defoliated. In the moist environment beneath plants the fungus penetrates pericarps and seeds. Green seed cannot be infected, even by wounding, until the pericarp becomes senescent, when rapid invasion and sporulation occur. The pathogen survives at least 3 years in seed. Infected seeds and volunteer or carryover plants supply initial field inoculum. Excellent control was achieved, with only 3.3 per cent average germination loss, by treating seed 30 minutes in water at 51.7° C. after a 1-hour soak in cool water. The disease was eliminated in extensive tests when treated seeds were planted in areas free of infected plants, and reduced when grown near infested areas by delayed infection. Seeds are easily indexed when held moist in glasshouse flats to determine infection.

Transmission of Rhizoctonia solani in Pepper Seed and Its Prevention. KENNETH F. BAKER. Pepper seedlings in field seedbeds and glasshouse flats usually sustain severe losses from Rhizoctonia pre- and post-emergence damping-off in southern California. This soil fungus developed in pasteurized soil planted with infected seed. Internal seed transmission was demonstrated by planting on moistened, sterile black peat in Petri dishes and by sectioning. Pepper fruits, particularly large types, frequently rest on the ground in seed fields and much Rhizoctonia "soil rot" occurs before the delayed harvest. The fungus grows into the seed from the funiculus in the micropyle-radical region. Maceration and fermentation in cleaning favor further infection. The fungus also is carried in small bits of fruit tissue. Up to 0.3 per cent of commercial seed is infected. This infection is important in (a) contaminating pasteurized or noninfested soil and (b) perpetuating strains causing damping-off, wire-stem, and fruit decay. Only certain strains infect the fruit and therefore become seed-borne. Treatment of the seed in water at 51.7° C. for 30 minutes eliminated the fungus without germination loss in extensive tests during three years. Nontreated seed produced 42.0 per cent fewer marketable plants in pasteurized soil than did treated seed. The treatment also controls similar infections of eggplant and zinnia seed.

Observations on the Root Endophyte, Rhizophagus, in Culture. J. T. BARRETT. There are many records of occurrence and study of the Rhizophagus type of endophytic fungi in roots of many plants. Of these none has reported success in establishing the

organism in culture although slight growth has resulted in a few cases. After several unsuccessful attempts the writer has obtained a culture of *Rhizophagus* from the root of garden pea. This was accomplished by placing a small piece of boiled hemp seed directly on a few hyphae attached to a piece of infected root in water. After a few days new growth was apparent. The piece of seed bearing the new growth was transferred to a Petri dish containing sterile tap water with a fresh half of boiled hemp seed submerged in the center. Within ten days mycelium bearing numerous typical vesicles had covered the bottom of the dish. Subsequently the fungus was established on water agar plus a small amount of water from autoclaved hemp seed and on other media. For the first time one species or strain of this interesting cosmopolitan root endophyte is made available for a better taxonomic and biological study.

Myrothecium Leaf Spot and Canker of Gardenia. J. T. BARRETT and DORIS ANN HARDMAN. In a previous report of this disease, in 1941, it was suggested that the causal organism was a new member of the family Tuberculariaceae. Subsequently the pathogen was identified as *Myrothecium roridum* Tode and our work furnishes the first and only report of its occurrence in California. The fungus is known now to attack also gardenia cuttings in rooting beds under high humidity and high temperature conditions. Successful infection of tomato plants with and without previous injury and of tomato fruit with needle punctures confirm in part work previously done in England. The disease is not widespread and apparently is easily prevented by using proper culture conditions.

Controlling Fruit Spoilage in Dates. DONALD E. BLISS. Disastrous crop losses of dates occur in California about one year in five. Moist weather during August and September is conducive to fungus spoilage (*Aspergillus niger* V. Tiegh., *Alternaria citri* E. and P. em. Bliss and Fawcett, etc.) and to insect infestation. A search was begun in 1940 for an effective protectant fungicide that would be easily removed from ripened dates and nontoxic to man. Among 11 fungicidal treatments tested in the field, significant differences in fungus spoilage of Deglet Noor dates were obtained as follows: in 1943, sulphur dust; in 1944, sulphur and a dust containing 5 per cent Fermate in sulphur; and in 1945 (a year of severe spoilage), Fermate sulphur mixture and a dust containing 10 per cent Yellow Cuprocide in sulphur. Whereas in 1945 untreated dates had 53.18 per cent fungus spoilage, those treated with Fermate-sulphur mixture had only 11.11 per cent. Fruit quality and palatability were not reduced. Date mites, *Paratetranychus simplex* (Banks), were controlled by the treatment, and relatively few dried fruit beetles, *Carpophilus hemipterus* L., were found. Residues of ferric dimethyl dithiocarbamate (Fermate) on treated dates were reduced to 4.90 p.p.m., and those of sulphur, to 11.82 p.p.m. by the Nussbaum cleaning method. Fermate-sulphur mixture is being tested commercially.

Sparse Leaf of Italian Prune. EARLE C. BLODGETT. A disease affecting several trees in one orchard in southwestern Idaho was observed first in 1942. The affected trees were characterized by, (1) a delay in blooming and leafing of 10 days or more, (2) sparse foliage confined largely to the periphery of the trees giving them an umbrella shape, and (3) marked reduction in fruitfulness although the fruit is of good size. The trees are not dwarfed. There is no good evidence of orchard spread. Leaf symptoms are expressed as small leaves with irregular margins and mild diffuse mottling. In general appearance some of the leaves are similar to those of cherry crinkle leaf. Bud inoculation tests indicate that sparse leaf is bud perpetuated but to date there is no proof of transmission.

Studies on Tip Burn of Sugar Beet and Lettuce. EUBANKS CARSNER. Sugar-beet tip burn develops on etiolated leaves in total darkness under conditions of high humidity. Recovery of plants so affected occurs in total darkness under relatively low humidity. Tip burn is more severe under low light intensity if the humidity is high. Lettuce tip burn is directly comparable to sugar-beet tip burn in regard to the relationships of light intensity, nitrogen nutrition, humidity, vigor of growth, age of plants, and recovery. The responses to environmental influences, both in showing symptoms and in recovery, are more rapid in lettuce than in sugar beet.

Phyllody of Common Beans, a Graft-Transmissible Disease. B. F. DANA. Scions from phylloid inflorescences of naturally infected bean plants were cleft-grafted on greenhouse-grown plants of Red Kidney, Bountiful, Asgrow Stringless Green Pod, and U. S. No. 5. Phyllody occurred in nine of ten inoculated plants, and scions from their phylloid inflorescences transmitted phyllody to 11 of 20 plants of Logan, Red Kidney, Bayo, Bountiful, and U. S. No. 5. No transmission was obtained by inserting wedges of tissue from phylloid plants into stems of 32 inoculates. Phyllody has occurred locally in East-

ern Oregon on varieties of common beans that are resistant to curly top as well as on plants of susceptible bean varieties that escaped infection. A similar disease, big bud, occurs on tomato; and what appears to be phyllody has occurred on soybean, lima bean, alfalfa and squash. Phyllody, apparently induced by aster-yellows virus, also has appeared on aster, scabiosa, delphinium, California poppy, chrysanthemum, marigold, cosmos, and carrot, which are known to be hosts of aster-yellows virus. This latter fact suggests that the simultaneous occurrence of phyllody symptoms on common bean, lima bean, and soybean, tomato, alfalfa, and squash may be due to infection by one or more strains of the aster-yellows virus.

The Spreading Factor in Plant Tissues. J. DUFRENOY. Hyaluronidase, the "spreading factor" affecting animal tissues, is prevalent in invasive bacteria, in the poisonous secretions of snakes or insects, and in the sperms of mammals. Plant pathogens spreading rapidly from the infection site, are apt to induce in cells at a distance a dissociation of the cytological constituents into nucleoproteins and fatty droplets, and an active secretion of phosphorylated compounds into the intercellular spaces. Pathogens unable to induce such changes in normal tissues may cause a spreading lesion only when and where a mechanical, physical, or chemical shock already had caused a breakdown of cell constituents. For instance, *Ovulinia azaleae* spots petals bruised by beating rains, by pushing hyphae between epidermal and subepidermal cells, which, as long as they survive, release compounds to the intercellular spaces. Pollen tubes, growing in the tissues of the style, behave as efficient parasites, in promoting the secretion of compounds from nearby living cells.

Reversible Sensitization of Plant Tissues Following Heat Treatment. J. DUFRENOY and H. S. REED. Heating plant tissues at 50–52° C., before inoculating with spores of pathogens, may lower the resistance, as observed by Edgerton *et al.* in stalks of sugar cane which received a hot-water treatment before being inoculated with conidia of the red-rot fungus. Conversely, heating after inoculation inactivates conidia so that they fail to develop a red-rot lesion; more important still, canes affected by chlorotic streak may be freed of the virus by the hot-water treatment. Heating at 50–52° C. causes a reversible swelling of mitochondria and thus a temporary dispersion of the complexes of nucleoproteins and lipids, now recognized as the cytological site of activity of dehydrogenases, whereas the copper proteins, acting as polyphenol-oxidases, are scarcely affected by temperatures below 60° C.; therefore, heating above 50° but below 60° C. causes a decompensation of respiration, as dehydrogenases are no longer apt to make hydrogen available as an acceptor to molecular oxygen, activated by oxidases. Heat treatment, properly applied, induces a shock from which cells recover, as mitochondria regain their normal appearance of rods or threads, staining sharply with reagents for phosphorus.

Some Studies on Curly-Top of Potatoes. N. J. GIDDINGS. Seedling potato plants of the variety Earlaine have been inoculated in the greenhouse with curly-top virus, and 43 per cent of the plants became infected. Infection was secured with 8 of the 10 virus strains used and with other virus selections which had not been designated as strains. The percentage of plants infected was greatest among those plants inoculated with the more virulent strains but one plant was infected by strain 7 which produces only very mild symptoms on sugar beet. Many of the infected plants did not yield any tubers but the tubers from 18 of them were saved and plants grown from them were tested. The 28 tubers from 16 seedling plants gave 26 plants in which the presence of curly-top virus was demonstrated and two in which it was not, while 25 tubers from 2 plants were all negative. Third generation plants were not tested. In commercial potato fields tests from plants with symptoms suspected of being curly top have been negative in every instance thus far. Ninety-one such tests have been made and include suspected material from Oregon, northern California, Bakersfield, Shafter, and Perris, California. Extensive inoculation experiments with different curly-top-virus strains have been made using White Rose, Katahdin, and Russet Burbank potato varieties. Very heavy inoculations have resulted in a small percentage of infection in each of the potato varieties both in the greenhouse and in the field. Field inoculations in replicated plots have thus far given no evidence of significant yield differences between the inoculated and the noninoculated plots.

The Comparative Value of Certain Organic and Inorganic Sulphur Compounds in the Control of Botrytis Blight of Tulips. C. J. GOULD. Bordeaux and certain other mixtures or compounds containing copper have been recommended for the control of tulip blight. (caused by *Botrytis tulipae*), but severe injury has often resulted from the use of these sprays. Since 1942 in field tests with 24 different sprays or dusts, certain organic sulphur compounds consistently have given excellent blight control with little or no burning. In order of decreasing control the sulphur compounds may be roughly arranged as follows: Fermate (ferric dimethyldithiocarbamate), Zerlate (zinc dimethyldithiocarbamate), Ter-

san (tetramethyl thiuram disulphide), Fermate-sulphur dust, zinc ethylene bisdithiocarbamate, Dithane (disodium ethylene bisdithiocarbamate), potassium resin polysulphide, and sulphur dust. Phenothiazone was promising in one year's test. The best material in each of the 5-year's tests was Fermate (2 lb./100 gal.).

Sodium Arsenite, a Promising Control of Dead-Arm of Grapes. WM. B. HEWITT. Sodium arsenite solution containing an equivalent of 3 lb. arsenic trioxide per 100 gal. water was sprayed on grape vines, variety Olivette blanche, infected with *Cryptosporella viticola* Shear during dormancy, not earlier than 3 weeks after pruning. Three blocks of vines, 6 rows each, averaging 43 shoots per vine, sprayed February 11, 1944, had an average of 4.9, 4.9, and 5.1 diseased shoots per vine; corresponding nonsprayed blocks had 27.0, 25.5, and 22.6 diseased shoots per vine. Similar blocks of vines sprayed in 1945 had very little disease. Shoot and leaf infections were light in 1946; however, in 3 blocks of vines sprayed on March 14 there were 2.6, 18.7, and 7.3 per cent of the vines with one or more diseased shoots and in corresponding nonsprayed plots 46.6, 27.5, and 52.6 per cent of the vines similarly diseased. The extrusion of spores from pycnidia on diseased spurs collected at random from the 1944 plots was as follows: 1–10 per cent of the pycnidia on 38 out of 138 sprayed spurs and 60–85 per cent of the pycnidia on 93 out of 106 nonsprayed spurs extruded spore horns.

Hydrogen Fluoride Injury to Prune Trees in Washington. FOLKE JOHNSON, V. L. MILLER, and D. F. ALLMENDINGER. Hydrogen fluoride, given off in smoke from aluminum factories, was found to produce considerable injury to Italian prune orchards in western Washington. The injury began as a marginal scorch of the leaves with necrotic areas appearing in the center of the lamina. This injury was followed by a severe leaf drop in mid-summer. The fluorine content of 52 samples of oven-dried prune leaves collected from localities where aluminum factories were located varied from 18 to 1400 p.p.m. In contrast, five leaf samples from a factory-free area had a fluorine content which varied between 6 and 15 p.p.m. Leaves with only a slight injury had a lower fluorine content than those with severe leaf spotting and marginal scorch, and the necrotic areas had a higher fluorine content than the green tissue. Similar symptoms were produced in peaches, plums, apples, pears, English walnut, Italian prunes, strawberries, and raspberries when subjected for eight days to a concentration of 5 p.p.m. hydrogen fluoride by weight, in a sealed chamber. Only doubtful symptoms were noticed in filberts and sour cherries at this concentration. Injury to prune leaves was also produced after treatment with several fluorine compounds used as sprays.

Fungicides for the Control of Brown Rot of Citrus. L. J. KLOTZ and G. A. ZENTMYER. Because of the aggrevation of HCN fumigation injury to citrus trees by prior coverage with copper sprays, pathologists have for many years sought for a non-copper spray material that would be effective against Phytophthora brown rot of citrus fruit. The search has been intensified during the past two seasons because in some localities citrus trees have been directly and severely damaged by copper sprays even in the absence of HCN fumigation. Many fungicides, including the new organic spray materials, are being tested. Results thus far show that no satisfactory substitute has been found. However, among those tested and found worthy of further study are: disodium ethylene bisdithiocarbamate with zinc sulphate and lime, tetrachloroquinone, zinc dimethyl dithio carbamate and 2,3-dichloro-naphthoquinone. While in laboratory experiments these materials have shown promise approaching the efficacy of Bordeaux mixture, in the grove they have failed to weather satisfactorily and to protect fruit from decay by the brown-rot fungi.

Tissue Relationships of Certain Dodders to Some Host Plants Used in Virus Disease Studies. C. F. LACKEY. Some dodders have been found useful in the transmission of certain virus diseases. Tissue relationships of three dodders have been studied on some of the host plants used in virus disease studies. The species used were *Cuscuta californica* Choisy, a very small dodder, *C. subinclusa* Dur. and Hilg., a larger one, and *C. americana* L., the largest of the three. All grow fairly well on sugar beets and tobacco, *Nicotiana tabacum. C. americana* grew best on *N. glauca.* There its haustoria do not pass through the xylem ring but make good connections with the external phloem. On *N. tabacum* it grows through the xylem ring into the pith, making only limited connections with the internal phloem. *C. californica* and *C. subinclusa* make but few connections with the vascular tissue of the cucumber and so do not grow vigorously on it. *C. subinclusa* makes no vascular connections with certain squash varieties and so does not grow on them. *C. californica* grows well on tomato, making good vascular connections. *C. americana* does not grow vigorously on tomato. Hypertrophy of cortex cells is induced which interferes somewhat with vascular connections between the dodder haustoria and the host. *C. subinclusa* will not establish itself on tomato, because mere contact of its

haustoria with tomato stems produces great hypertrophy of the cortex cells in the region of contact. Further growth of haustoria to apparently inhibited and enlarged host cells prevent haustoria reaching the vascular tissue. Lack of vascular connections between dodder and host would prevent transmission of phloem-limited viruses such as the curly-top virus.

Localized Chemical Applications to the Soil and Their Effects upon Root Rots of Beans and Peas. LYSLE D. LEACH and WILLIAM C. SNYDER. The prevention of bean root rot (*Rhizoctonia solani, Fusarium solani* f. *phaseoli. Thielaviopsis basicola*) and pea root rot (*R. solani, F. solani* f. *pisi, Ascochyta spp.*), hitherto largely dependent only upon cultural practices (crop rotations and cover cropping), has been attempted by chemical soil treatments. Applications of chemicals in the row when seeding, similar to the formaldehyde-drip treatment against onion smut, have been made in artificially infested soils in the greenhouse and in naturally infested soils in the field. Of the materials so far used, 25 per cent disodium ethylene bisdithiocarbamate (Dithane D14) has proven most effective. When applied at the rate of 1 gal. per acre, or 2 lb. of the dry powder (Dithane A10) per acre, marked reductions in the degree of root-rot infection were obtained. It has not been determined whether the action is directly fungicidal or fungistatic, or indirect through its effect upon the host. Materials of this kind applied locally as a soil treatment in the planted row, on or with the seed, seem to offer a promising approach to the control of root-disease complexes of a variety of crops.

The Effect of Terminal Smut Galls on Yield and Seed Grade of Detasseled Hybrid Corn. J. D. MENZIES and C. O. STANBERRY. Galls of common corn smut frequently occur at the top of corn stalks that have been detasseled for hybrid seed production. These galls result in yield reduction which varies directly with the size of gall. In this study of 864 smutted plants, galls under 2 inches in diameter reduced the yield 8.8 per cent, while galls of 2 to 3 inches in diameter and over 3 inches in diameter reduced yields 14.1 and 40.1 per cent, respectively. Yield reduction resulted both from smaller ears and fewer ears per stalk. This effect was accompanied by smaller-sized kernels and a slight increase in cull grade. The average yield loss per plant in marketable grain due to these terminal smut infections was calculated to be 22 per cent. Smut did not affect the germinability of the seed.

Root Rot of Condiment Sage. JOHN T. MIDDLETON. A disease affecting the root, crown, and stem of mature sage, *Salvia officinalis*, was observed in the Imperial Valley of California in a planting grown for seed. Diseased plants are stunted, wilted, and the leaves ash grey-green in color; badly diseased plants do not produce inflorescences and usually die. Infected fibrous roots are discolored, the cortex water-soaked and flaccid. Longitudinally oriented, sunken, dark brown to black, lesions that may girdle the root, frequently occur in the larger secondary and primary roots. Infection usually progresses upwards, causing cortical necrosis and discoloration of the outer portion of the stem stele; lesions may extend 2 to 4 inches above the soil. The disease is most conspicuous where plant beds are low, irrigation water stands, and drainage is poor. Isolations from diseased material collected in February, 1946, yielded *Phytophthora parasitica*, while those made from March collections yielded *P. parasitica* and *Pythium aphanidermatum*; only *P. aphanidermatum* was secured from May samples. The pathogenicity of each of the two fungi to sage has been demonstrated by artificial inoculation. Inoculations with mixed cultures neither depressed nor accelerated disease development. Symptoms produced by either pure or mixed cultures of the fungi are similar to symptoms on naturally infected plants.

The Production of Ascochyta-Free Pea Seed in Southern California. JOHN T. MIDDLETON and W. C. SNYDER. Pea seed devoid of *Ascochyta* has been produced from both naturally and artificially infected seed when grown in the Imperial and Temecula Valleys of southern California. Naturally infected Alaska, naturally and artificially infected Little Marvel, and artificially infected No. 76 seed have been used. Peas at Temecula were grown during the summer rain-free period, while those in Imperial, near Brawley and Calipatria, were grown during the winter and late spring. The average rainfall at Brawley for the 5-month fall period, August through December, totals 1.19 inches, while that for the corresponding spring period, November through March, totals 1.64 inches; the annual rainfall is 2.43 inches. At no time throughout the three-year period of study have aerial symptoms of Ascochyta blight been observed in any of the plantings. Seed harvested from plants grown from infected seed have been proved to be free of *Ascochyta*. Other seed-borne diseases have likewise not been observed on aerial portions of the plant. Plants grown from healthy pea seed sown in land cropped to peas for the past 23 years had neither below- nor above-ground symptoms of Ascochyta blight.

Sieve Tube Necrosis in Orange Trees Affected by Quick Decline During the Spring Season. HENRY SCHNEIDER. In the spring of 1946 the necrosis of sieve tubes of the sour orange stock of sweet orange trees, at and below the bud union, was similar to that found in the winter. In the process of necrosis, callus forms on the sieve plates; then the sieve tubes and companion cells are crushed by the expansion of the adjacent parenchyma cells, which are occasionally divided and usually cleared of fat globules and starch. Wound gum was not found. The following stages of necrosis have been found: (1) Those in which necrosis was present a short distance below the bud union. Contrary to normal obliteration, which sometimes appeared earlier in sour than in sweet orange, necrosis usually occurred first in the youngest or medium-aged sieve tubes in trees having no top symptoms. (2) Those in which the older, outer sweet orange sieve tubes above the union had also become necrotic, apparently because of the girdling effect. (3) More advanced stages in which the cambium had produced new phloem. In these cases a narrow cylinder of functioning phloem was usually present below the union in the sour orange stock and a wider one above the union; but at the union the sieve tubes were callused or necrotic. In 3 trees out of 10 that were in the equilibrium stage when sampled in June, there were a few functioning sieve tubes near the cambium at the union.

Newly Discovered Leafhopper Vectors of California Aster-Yellows Virus. HENRY H. P. SEVERIN. Nineteen newly discovered leafhopper species, and three species and one biological race previously reported, are vectors of the virus as follows: Short winged and long-winged (biological race) aster leafhoppers, *Macrosteles divisus* (Uhler). Phlepsius group: *Paraphlepsius apertinus* (Osborn and Lathrop), *Texananus lathropi* Baker, *T. pergradus* DeLong, *T. spatulatus* Van Duzee, *T. oregonus* Ball, *T. latipex* DeLong. Thamnotettix group: *Idiodonus heidemani* (Ball), *I. kirkaldyi* (Ball), geminate leaf hopper, *Colladonus geminatus* (Van Duzee), mountain leafhopper, *C. montanus* (Van Duzee), *C. commissus* (Van Duzee), *C. flavocapitatus* (Van Duzee), *Friscanus intricatus* (Ball), *F. rupinatus*, *Acinopterus angulatus* Lawson. *Gyponana hasta* DeLong, the first reported vector in the subfamily Gyponinae. *Cloanthanus irroratus* (Van Duzee), *C. dubius* (Van Duzee). *Euscelis maculipennis* DeLong and Davidson, *Fiebriella florii* (Stal). *Chlorotettix similis* DeLong. Twenty-two leafhopper species and one biological race belonging to twelve genera have been demonstrated to be vectors of the virus.

Transmission of Virus of Pierce's Grapevine Disease by Spittle Insects. HENRY H. P. SEVERIN. Four species and six varieties belonging to three genera are vectors of the virus as follows: *Aphrophora angulata* Ball, *A. permutata* Uhler, *Clastoptera brunnea* Ball, *Philaenus leucophthalmus* var. *leucophthalmus* (Linnaeus), *P. leucophthalmus* var. *pallidus* (Zetterstedt), *P. leucophthalmus* var. *fabricii* Van Duzee, *P. leucophthalmus* var. *marginellus* (Fabricius), *P. leucophthalmus* var. *spumarius* (Fallen), and *P. leucophthalmus* var. *impressus* DeLong.

Studies of a Bud Failure Condition in Almond Trees. GILBERT L. STOUT and E. E. WILSON. A disorder in almond trees, the chief character of which is failure of many buds to live and develop into new growth, occurs in many orchards in north central California. The bud failure often results in a peculiar type of twig and limb development and in disorderly arrangement of twigs and branches, suggesting the term "crazy top." Other symptoms include dying of twigs, delay in blooming, sparsity of foliage, and, in the Peerless variety, brown necrotic areas on twigs produced during the previous season. These areas later become rough and cracked. In both Nonpareil and Peerless trees, bands of rough bark up to a foot in width develop on older wood. Buds and scions from affected Nonpareil and Peerless trees failed to transmit the disorder to normal Nonpareil, Peerless, and Drake trees within five years, but a high percentage of buds and scions from Nonpareil and Peerless perpetuated it. Scions from healthy Nonpareil placed in affected Nonpareil have remained normal after eight years. A bud failure of Drake similar in some respects to that in Nonpareil and Peerless has been transmitted with scions to Nonpareil within two years. This suggests that the disorder in Drake may be different from that in Nonpareil and Peerless.

An Electron Microscope Study of Two Strains of Potato X Virus. WILLIAM N. TAKAHASHI and T. E. RAWLINS. Larson's potato virulent ring-spot and potato latent-mottle viruses were studied with the electron microscope. They were found to be indistinguishable in form and size. A high proportion of the particles have lengths between 500 and 600 mμ. The particles lack the rigidity characteristic of tobacco-mosaic virus and are often curled into various forms. The similarity in appearance of the two strains of potato X virus is in agreement with the idea that mutations are frequently not accompanied by detectable changes in the appearance of the virus particle.

An Electron Microscope Study of Mutation in Tobacco-Mosaic Virus. WILLIAM N. TAKAHASHI and T. E. RAWLINS. Common tobacco mosaic virus and a yellow strain isolated from it were studied with the electron microscope. The yellow strain was found to be indistinguishable in size and form from the parent strain. It is concluded that mutation in this case was not accompanied by a modification of the virus particle sufficiently great to be detected by the electron microscope.

The Use of Triethylene Glycol Vapor as an Aid in the Control of Air-Borne Contaminants. W. J. VIRGIN and JEAN C. MALOIT. Considerable trouble is often experienced in pathological laboratories with air borne contaminants when doing various types of cultural work. Steam is often employed but it has definite disadvantages. The use of triethylene glycol vapor has proved very effective in eliminating air-borne con taminants and has no uncomfortable effect on the worker. A beaker containing 100 cc. of triethylene glycol placed on a low flame of a Bunsen burner will effectively fume a room of 7,000 cu. ft. in about 20 min. and will use approximately 40 to 50 cc. of liquid. Smaller rooms will take proportionately less. The liquid becomes discolored on heating but this has no effect on its ability to vaporize. The vapor rises to the ceiling and gradually settles down to the floor. It appears that as the vapor settles it carries down with it most of the organisms that may be floating around in the air. Exposing Petri dishes containing agar for 5 min. before and after fuming showed that there was considerable reduction in the number of air-borne contaminants after fuming as compared to before fuming.

Copper Carbonate for Chestnut Root Disease. WILLIS W. WAGENER and JAMES W. KIMMEY. In the summer of 1942 there appeared in an 80-year-old planting of European chestnut a crown rot, apparently caused by *Phytophthora*, but attempts to isolate the causal organism have been unsuccessful. In May, 1943, twelve trees were treated by a method reported by Landaluze in Spain. The soil was excavated from around the bases of the trees, records were made of dead areas present, and the cleaned bark was painted with a suspension of copper carbonate in water to which ½ lb. per gal. of casein glue had been added as an adherent. The soil was then refilled around the trees. Three trees, 70 per cent or more girdled by the disease when treated, later succumbed or became completely girdled; three others showed small extensions of killings; and the remaining six suffered no further injury. While the treatment did not fully arrest the disease, it showed evidences of some beneficial effect. On this basis and that of the promising results from somewhat similar treatments reported from Spain, it is believed that additional trials of the method here are warranted where this type of crown rot becomes active.

The Vertical Dispersion of Spores in the Air Near the Ground by Winds of Low Velocity. E. E. WILSON. During overcast periods when thermal turbulence was weak and probably absent the comparatively large (33 μ) spores of *Lycopodium* were carried upward when released 3 ft. from the ground into air moving 0.6 to 5.4 m.p.h. Judging from the ratio of the aerial density of the spores above the level of release to the density at the level of release, vertical dispersion per unit of downwind distance was greatest at the lowest wind velocity, and diminished rapidly as the velocity increased. A similar trend was apparent on sunny days when some thermal turbulence probably existed. Ratios pertaining to dispersion downward from the 3-ft. level likewise diminished as air movement increased. On an overcast morning and with wind velocity 1.5 m.p.h., spores were carried to earth from a height of 3 ft. within a distance of about 12 feet. The number intercepted by the ground increased from this distance to about 20 feet and declined thereafter.

Influence of Host, Fungicide, and Pathogen on the Utility of a Spreader in Fungicide Tests. C. E. YARWOOD. In greenhouse tests the addition of 0.05 per cent phthalic glycerol alkyl resin spreader to bluestone spray decreased its injury on bean, cucumber, and mustard foliage but increased its injury on pea foliage; increased its eradicant (therapeutic) action for bean powdery mildew and cucumber powdery mildew and for bean rust and sunflower rust; increased the protective action of Bordeaux and other insoluble coppers for bean rust, bean powdery mildew, snapdragon rust, onion downy mildew, and cucumber downy mildew; increased the protective value of lime-sulphur for onion downy mildew and snapdragon downy mildew; but decreased the protective value of lime-sulphur for bean powdery mildew, and cucumber powdery mildew, and of Fermate (ferric dimethyldithiocarbamate) for snapdragon rust. The ratio of the LD95 of fungi-" cide sprays without spreader to the LD95 of the same fungicide with spreader ranged from 1: 4 with lime-sulphur as a protective spray for cucumber downy mildew to 455: 1 with Bordeaux as a protective spray for snapdragon rust.

Yield from Potato Giant Hill. C. E. YARWOOD. In the San Juan Bautista district of California the dying of Netted Gem potato plants at 70 to 100 days from planting has

been associated with infection by *Verticillium alboatrum*, *Rhizoctonia solani*, *Phytophthora infestans*, and *Alternaria solani*, the first being most important. Giant Hill vines occurring naturally in plantings of certified seed have lived up to 135 days and have been less severely infected with or injured by these fungi. Giant Hill selections yielded less than normal vines up to 90 days from planting, but yielded 35 to 63 per cent more weight of tubers at 120 to 135 days from planting in 1942 to 1945. At Half Moon Bay, Giant Hill plants yielded 30 per cent more than normal at 106 days from planting in 1944, but normal plants yielded more than Giant Hill at Stockton and Berkeley in 1945. Giant Hill tubers were larger, had more overgrowths, deeper eyes, and had a tendency to be spindle-shaped, but at maturity were similar in cooking quality to normal tubers. Strains of Giant Hill differed with respect to longevity of vines, length of vines, susceptibility to early blight, yield of tubers, and shape of tubers.

Black Canker of Cherry. S. M. ZELLER, J. A. MILBRATH, and J. R. KIENHOLZ. Black Canker of Napoleon sweet cherry has been found in Polk, Union, Wasco, and Washington counties, Oregon. Cankers start in one-year-old twigs, first as slightly swollen areas in which the bark splits lengthwise. These areas grow into rough black cankers the ultimate size of which is more or less determined by the size of the affected branch. Some infected trees are very severely cankered, while others may have few cankers. No abnormal fruit or leaf symptoms have been observed. Many attempts to isolate a causal organism have failed, but transmission of the disease was brought about by graft inoculation after 2-year incubation.

The Rôle of Microorganisms in Avocado Tree Decline. GEORGE A. ZENTMYER and L. J. KLOTZ. Avocado decline has been recognized occasionally in California for many years but only recently has assumed considerable proportions. Poor drainage of the soil, occurring either on very heavy soils or on soils with an impervious layer near the surface, generally initiates the trouble. A specific biological factor or factors may also be involved. Avocado seedlings have made greater (3–4 times) and more vigorous growth in sterilized (autoclaving or chloropicrin) soil than in nonsterile soil from "decline" areas. At least two factors appear to be involved in decline: waterlogging and *Phytophthora cinnamomi*. When present, *P. cinnamomi* may accelerate decline, but decline occurrence is not dependent on the presence of this fungus alone. Injury will occur from waterlogging periods of 10–14 days or more in soils which have been sterilized with chloropicrin or by autoclaving. Inoculation of soil with *P. cinnamomi* has resulted in injury to the root system even when excess water was not present. Toxic products formed by bacteria and other microorganisms under anaerobic conditions, including butyric acid, nitrite, and H_2S, also play a part in decline. Such products are increased and consequently root injury is greater in soils in which the carbohydrate content has been increased (addition of sugar).

PHYTOPATHOLOGICAL NOTES

Injury to Plants by Minute Amounts of 2,4-Dichlorophenoxyacetic Acid.[1]—Many new weed killers containing salts or esters of 2,4-dichlorophenoxyacetic acid, apparently effective for the purpose intended, are now available. It appears, however, that they must be used with extreme care to avoid injury to valuable plants. Unintentional injury may be caused to sensitive plants in at least 4 ways: namely, from toxic residues left in spray or dust-mixing equipment, not removable by the usual methods of cleaning or washing; vapors from equipment and containers; absorption by roots; and drift of spray or vapors by air currents. These conclusions are based on observations of accidental injury and on laboratory tests.

Following use with 0.1 per cent ethyl ester of 2,4-D, a galvanized iron hand sprayer was washed 3 times with hot water and soap, then rinsed several times with hot water. A few weeks later rose, zinnia, and phlox were sprayed with an insecticide from this sprayer. Some time later the new rose shoots were spindling and the leaflets were narrow, deeply toothed, curled, and often mottled. The young leaves from the terminal buds of phlox were narrow and mottled and had irregular margins. Numerous shoots that developed in the axils of the abnormal phlox leaves often were of jack-in-the-pulpit type. The apical leaves of zinnia were very narrow and yellow and usually died. The leaves of shoots that developed in the axils of uninjured leaves of zinnia appeared normal.

In an experimental greenhouse, potted tomato and cabbage plants were injured by what appeared to be a volatile material in the air. The upper leaflets were narrow and rolled and the leaves bent downward along the curved stem. These abnormalities resembled the early symptoms of 2,4-D injury on rose and phlox; therefore, it was suspected that injury to tomato and cabbage was due to fumes of 2,4-D from a wheelbarrow sprayer placed in the greenhouse after a 0.1 per cent butyl 2,4-D had been used in it. One lot of potted tomato plants from this greenhouse, transplanted to the field 2 days after the sprayer had been placed in the house, appeared normal when set in the field; but symptoms of injury appeared later. Another lot of potted tomato plants from this greenhouse, transplanted to the field before the sprayer had been placed in the house, developed normally without injury of any kind.

To test whether fumes of butyl 2,4-D injured tomato and cabbage, and to eliminate other gases as a cause of injury, the sprayer was placed in an open insectary. A tomato and a pepper plant, placed in the open tank, developed leaf rolling and drooping in 12 hours. The sprayer was then moved to another building. Tomato, cabbage, pepper, zinnia, petunia, tobacco, and *Nicotiana rustica* developed leaf rolling and drooping after 1

to 2 days when set in a closed room at some distance from the open spray tank.

In addition to leaf rolling and drooping, tomato, tobacco, and petunia plants, after several days' exposure to fumes of 2,4-D developed raised, white galls on the upper swollen stems and petioles. Root initials formed on these galls in a moist atmosphere. In tomato gradually the epidermis died and peeled off, causing the upper leaves to wither. In some cases, in a moist atmosphere, guttation was prominent on many leaves and sometimes water droplets oozed from the stems as the epidermis cracked. Several tomato plants injured by fumes of 2,4-D produced seedless fruits. Numerous shoots formed at the base of the tobacco plants.

An attempt was made to remove toxic residues of butyl 2,4-D from the wheelbarrow sprayer by successive washings with a saturated solution of trisodium phosphate, 1 part concentrated hydrochloric acid to 5 parts of water, and strong soap and water. After each washing, zinnia, petunia, cabbage, and *Nicotiana rustica* plants, exposed to fumes from the sprayer, were injured.

A 0.1-per cent emulsion of butyl 2,4-D was sprayed on experimental grass plots in March. In mid-May, in a nearby fence row and door yard, there were abnormal secondary shoots of rose, grape, hackberry, mulberry, locust, oak, redbud, mock orange, Virginia creeper, and pokeweed. The early growth on all of these species was normal. Most of the injured shoots were some distance above the ground and several feet from where the chemical was applied. On grapevines, 50 feet away, and on roses, at least 25 feet away, there were marked malformations and mottling. It is believed that vapors of 2,4-D, rather than drift of the chemical at spraying time or washing of the material to the roots and later absorption, were responsible for injury to these plants both because of the time elapsing between application and injury and because of the distance of the injured plants from the point of application. It appeared unlikely that 2,4-D could have washed to the roots in this area, although vapor may have drifted at the time of application. These observations indicate that this method of weed control in gardens and lawns must be used with care to prevent drift of the chemical or vapors to valuable plants, especially if the highly volatile compounds are used.

Laboratory tests with the ethyl and butyl esters of 2,4-D indicate that toxic residues of both esters may remain in metal spray equipment after successive washings in hot water and soap, and strong alkali. Two washings of metal sprayers with acetone apparently removed most of both esters; however, some injury still occurred to zinnia, petunia, and *Nicotiana rustica* when these plants were placed under a bell jar with one of the sprayers and to tomato plants sprayed with water from the sprayers.

Both esters caused injury to tomato, cabbage, petunia, *Nicotiana rustica,* and tobacco when either concentrated solutions or dilute emulsions were placed under bell jars with these plants. These tests indicate that it is diffi-

cult to remove toxic residues of the esters of 2,4-D from spray equipment and that volatile products from equipment and containers may injure sensitive plants in closed rooms or greenhouses. It is doubtful whether power equipment could be washed sufficiently by the usual methods to make it safe for other uses.

Three washings with tap water appeared sufficient to remove toxic residues of both the ammonium and sodium salts of 2,4-D from metal sprayers. There appeared to be no toxic volatile products from either the dry chemicals or solutions of the ammonium or sodium salts of 2,4-D because sensitive plants exposed to the dry salts and solutions for 5 days under bell jars were not injured.--E. M. JOHNSON, Kentucky Agricultural Experiment Station.

Gaseous Sterilization of Biological Materials for Use as Culture Media.— The ordinary procedure of sterilizing biological materials by heat has the disadvantage that it sometimes alters drastically the physical-chemical nature of the substance being sterilized. In addition there are frequently inconveniences attendant upon heat sterilization.

In order to circumvent these disadvantages as far as possible and to approach more nearly the need for sterile natural media for the purposes of isolating and culturing fungi,[1] both ethylene oxide and propylene oxide have been tried as sterilizing fumigants in place of heat. Their successful use over a 2-year period has prompted this note.

Although the use of these fumigants as insecticides is well known, it was the work of Whelton *et al.*[2] which suggested to the writers that such chemicals might be used for the preparation of sterile natural media. These investigators were confronted with the comparable problem of sterilizing dehydrated fruits in a manner which would leave the product in as natural a condition as possible, free from the caramelization and unnatural odors and flavors that attend heat pasteurization, and free from toxic residues which may result from sterilization.

The material to be sterilized, whether it be alfalfa hay, pea pods, dry bean straw, wheat seed, youngberry fruit, fresh carrot, dehydrated fruits and vegetables, etc., insects, or even soil mixtures, is placed in a container such as a fruit jar and if very dry is moistened slightly by lightly atomizing it with water or by placing a piece of wet blotting paper in the container. The fumigant (propylene oxide) is measured approximately in a graduated cylinder or pipette and introduced into the jar at the rate of about 1 cc. per liter capacity of the container. The cover is immediately screwed down over the mouth of the jar with a gasket to make it air-tight. The jar is shaken and put aside overnight or longer, at room temperature. The lid may then be loosened and the fumigant allowed to escape, after which the material is ready for use. Media for multiplication of inocula may be prepared in a

[1] Snyder, William C., and H. N. Hansen. Advantages of natural media and environments in the culture of fungi. Phytopath. 37: (in press). 1947.
[2] Whelton, Rita, H. J. Phaff, and E. M. Mrak. Control of microbiological food spoilage by fumigation with epoxides. Food Industries 18: 23–25; 318–320. 1946.

similar way. The dosage indicated here is probably excessive and no doubt could be cut down considerably for most materials. Ethylene oxide because of its low boiling point cannot be handled satisfactorily in this way at room temperature without special equipment.

In the preparation of natural media for culturing organisms the treated material may be combined directly with liquid or solid media, such as agar, or used alone. For example, in the preparation of a Petri dish of pea-straw agar a little of the ground-up, fumigated pea straw is transferred with sterile forceps from the jar to a sterile Petri dish. Over the straw is poured 10 or 15 cc. of melted $1\frac{1}{2}$ or 2 per cent water agar and the dish is then agitated in order to distribute the material evenly throughout the agar. The warm agar instantly drives off any residual fumigant which may still be present in the straw, whereupon organisms may then be cultured upon the medium as soon as it hardens. Where not combined with melted agar, additional time or warming may be required to dissipate residual gas from the straw.

Materials so fumigated, if not too moist, may be kept indefinitely in the closed containers without loss due to spoilage. Some plant materials prepared 1 and 2 years ago are still being used in this laboratory without resterilization.

Apparently no residue toxic to the growth of fungi is left in the tissue after sterilization and after dissipation of the fumigant. Furthermore, medium prepared from fumigated straw, for example, has proved superior to medium which has been made by autoclaving the pea straw with the water agar, probably because the nutrient contained in the straw largely remains in the pieces of tissue, apparently in a natural condition, and does not escape into the agar itself to any appreciable extent. This is a distinct advantage in that contaminants establish themselves with greater difficulty on a nonnutrient agar, whereas the fungi established in invaded substrates plated on this medium grow into the agar and reach the nutrient-containing pieces of pea straw.

What has been said here of pea straw applies likewise to other kinds of organic matter. Some 50 different kinds of plant and animal tissues, including scale and other insects, have been used in this way. Where fruiting of fungi is desired, the fumigated organic material may be sprinkled on the top of the water agar or more of it added at the time of pouring the plate.

Ethylene oxide because of its lower boiling point (10.7° C.) may best be handled in a low-temperature room. It is dissipated more rapidly from the material than is propylene oxide (boiling point, 33.9° C.) and is perhaps somewhat more efficient as a fumigant. However, since it is explosive in mixtures with air and more hazardous and difficult to use in the laboratory, the writers have preferred to use propylene oxide. Although inflammable, propylene oxide is easily handled. A small amount of it is kept in a stoppered bottle in a refrigerator held at about 7° C., ready for use at any time.

Gaseous sterilization has made it possible to prepare quickly and conveniently a variety of natural media without alteration by heat. The materials are collected and fumigated when available and stored in the jars until needed. The sterilization of limited volumes of soil, and of cultures of pathogenic organisms prior to their discard also has been carried out advantageously by this method. That soil may be sterilized with ethylene oxide has been reported by Roberts et al.[3]—H. N. HANSEN and WILLIAM C. SNYDER, Division of Plant Pathology, University of California, Berkeley.

D-D Mixture as a Soil Treatment for Bacterial Wilt on Tobacco.[1]—Commercial D-D mixture (dichloropropane and dichloropropene) was tested for control of bacterial wilt (*Bacterium solanacearum* E. F. Smith) of tobacco. The plots were 12 × 24 feet with water-tight sidewalls as described previously.[2] The material was placed in holes opened with a spade in the prepared soil March 8, 1944. No paper or water seal was used, although rain occurred on March 9 in an amount sufficient to wet the upper 2 inches of soil. A wilt-susceptible strain of tobacco was transplanted June 1, 1944.

Treatments at the rates of 0, 2, 4, and 8 ml. per square foot of soil surface had 82, 59, 24, and 0 per cent wilt, respectively, at the end of the season in 1944. The same plots replanted in susceptible tobacco without further treatment had 83, 71, 73, and 11 per cent wilt August 1, 1945. No toxic effects were observed although a dark green leaf color, brittleness, and upward roll of leaf margins typical of excessive chlorine occurred in 1944 in plots treated with the heavier rates.

D-D mixture gave considerable protection that persisted into the second successive tobacco crop as shown by the reduced amount of wilt in 1945 on the plot treated with 8 ml. per square foot. Some residual protection has been observed on soil treated with chloropicrin or urea, and on plots rotated to corn or other crops. However, the degree of protection from D-D mixture the second season was greater than with other treatments.—T. E. SMITH.

▸ * [3] Roberts, James L., L. E. Allison, Paul S. Prickett, and K. B. Riddle. Preliminary studies on soil sterilization with ethylene oxide. Jour. Bact. **45**: 40. 1943.

[1] Cooperative investigations of the Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, the N. C. Agricultural Experiment Station and Department of Agriculture.

[2] Smith, T. E. Control of bacterial wilt (*Bacterium solanacearum*) of tobacco as influenced by crop rotation and chemical treatment of the soil. U. S. Dept. Agr. Circ. 692. 1944.

POTATO DISEASES AND HYBRIDIZATION

T. H. Thung

INTRODUCTION

Economically the most important diseases of the potato crop in Java are the *Phytophthora* and wilt diseases. The latter, caused by *Bacterium solanacearum*, was known from different parts of Java long before the first was known. Reports about severe wilt were made by van der Goot (8) in 1924 and by Buijze (2) in 1929. The Phytophthora disease or blight has been reported from Java only since 1936 (5, 9); from that year onward losses due to this disease have been great. The statistical data indicate an important reduction in potato production; the production of Java in 1935 was 62,700 tons; in 1936, 1937, 1938, 1939, and 1940 it amounted, respectively, to 59,200 tons, 46,700 tons, 38,800 tons, 42,700 tons, and 40,500 tons (20). This reduction can only be explained as being due to losses caused by Phytophthora attacks. Many of the formerly well-known local varieties such as Preanger muis or Tenger muis, kentang wernie, hoei bien, and Franschen have disappeared because of their great susceptibility. More resistant varieties such as Eigenheimer and Bevelander are planted; and spraying with Bordeaux mixture is necessary to reduce the losses from *Phytophthora infestans*. Spraying makes the growing of the crop more costly but gives no absolute guarantee for a good yield, since all the known potato varieties are susceptible to wilt as well as to *Phytophthora*.

The wilt disease or brownrot causes not only damage to the stem but also a rot of the tuber; during storage of the potatoes great losses from tuber rot are common. The causal organism living in the soil remains virulent under favorable circumstances for several years. The possibilities of growing potatoes in noninfested soils are few, unless crop rotation is practiced and there are long periods without susceptible plants. Since many plants belonging to different families are host plants of *Bacterium solanacearum* (4) it is rather difficult to avoid reinfestation by the bacterium, susceptible plants in adjacent fields being a source of infection. Common crops such as tobacco, peanut, tomato, *Ricinus*, and beans are mentioned as host plants. Soil disinfection with sulfur, as practiced by Eddins (4) in Florida, gave no results in the Java soils (6), because of the difficulty of bringing these soils to such an acidity that *B. solanacearum* would be eliminated; even quantities as large as 1000 kilo sulfur powder per hectar (6) and 1 kilo per sq. m. (9) were insufficient. Wet rice cultivation has been observed to favor the elimination of soil infestation. Two reasons can be given: Firstly, an amelioration of soil structure can be effected by irrigation of fields during rice culture especially with water coming down from young volcanic areas and containing much silt. After the rice harvest the soil has to be tilled well and

373

dried. This amelioration, resulting in finer crumb texture and better aeration of the soil, favors the crop following the rice and does not favor the bacterium. Secondly, the antagonistic action between different soil microorganisms will be activated by irrigation. Thung (24) observed that *Protozoa* and many other organisms of wet fields reduce the numbers of *Phytophthora* and also of *Bacterium solanacearum*. Unfortunately the fields used for potato culture in Java are generally not to be irrigated because of lack of water nearby.

Desiccation of soil, too, may be an important control method. Nakata (11), studying tobacco wilt, found that a low water content was fatal to *Bacterium solanacearum* in several kinds of soil. By keeping the moisture of soil under this amount the bacterium may die out; this moisture level is low in sandy soil and high in clay soil. He reported that "the organism stops growth and gradually dies out when water content of soil reaches detrimental water content" of the organism, which corresponds with the "wilting coefficient" of Briggs and Schantz and "critical content residue" of Livingstone and Kokatsu for higher plants. Further he found that *B. solanacearum* grew only in soils with a pH between 6.1 and 8.1 and that "the organism is inhibited for its growth and finally dies out when cultured in the presence of *Aspergillus Oryzae*, *Actinomyces rutgerscens*, *Act. Californicus*, *Act. violaceus-ruber*, *Bac. mycoides*, *Bac. fluorescens*, *Bac. cereus*, *Bac. proteus*, and *Azotobacter chroococcum*." He advised: " (1) soil should be dried to reduce its water content to the detrimental water content of the organism, (2) the reaction of the soil should be adjusted not to keep pH 6.1 to 8.1 or (3) the soil should be inoculated with soil organisms inhibitory to the organism."

For the wet mountain climates of Java it is rather difficult to maintain this detrimental water content to such a depth that the soil will be freed of *Bacterium solanacearum*. In Java *B. solanacearum* tolerates a greater acidity *viz.* pH 4.5 (6); and sulfur in large quantities did not lower the acidity to such a degree as to eliminate *B. solanacearum*. The alkalinity of the soil can be heightened by lime supplements, but in practice this has not satisfactorily reduced wilt disease in the potato crops.

The antagonistic action of different microorganisms is to be stimulated by organic manures. Van der Poel (13) found a diminution of wilt disease in tobacco by supplying "katjang boengkil" (cake of ground peanuts after oil extraction) and ground parts of *Mimosa invisa*. But experiments with the same methods with potato in Java did not give satisfactory results (6).

The situation at present is that potato growing in Java is rather a risky affair. The production is not more than about 45 to 50 q. per hectar (20); in the temperate zones this production can be 4 to 6 times more (1, 12), partly because the potato grows better in the longer days of the temperate zone and partly because disease losses are less.

The possibility of finding resistant potato varieties has been studied and considerable work has been done already as regards resistance to *Phytoph-*

thora. A brief account has been given by Roemer, Fuchs, and Isenbeck (17).

HYBRIDIZATION

In breeding for resistance against *Phytophthora, Solanum tuberosum* has been crossed with wild immune varieties. Müller (10) found the so-called W-races of potato, which proved to be resistant against different physiological races of *Phytophthora infestans*. But, unfortunately, since 1932 a new race (Phytophthora race S) causes great damage to these W-races.

Crosses between *Solanum demissum* (a resistant wild variety with 72 chromosomes) and *Solanum tuberosum* are being studied by several institutes. Reddick (15) and Schmidt (see 3) use successive backcrosses with *S. tuberosum,* and Salaman (18) uses selfed progenies of his crosses *S. demissum × S. tuberosum.*

A loss of resistance of *Solanum demissum* was observed with successive backcrosses to *S. tuberosum* (3). Crosses with other wild species such as *S. andigenum, S. semidemissum,* and *S. antipoviczii* (3, 7, 19, and 21) have been made.

Although many promising results have been secured, until now no commercial variety has been obtained from interspecific hybridization. Increased resistance found in some varieties of *Solanum tuberosum* has been obtained by Stevenson *et al.* (23) and by Reddick (14, 16) in crosses between varieties of *Solanum tuberosum.* Chippewa has been crossed with Katahdin (both commercial varieties), and Ekishirodzu, a Japanese resistant variety, with Evergreen. Promising seedlings came from a cross between the varieties No Blight and Katahdin (22). Stevenson *et al.* (23) mentioned also selection against *Phytophthora* by selfing a susceptible variety, by crossing a resistant variety and a susceptible one, and by crossing susceptible varieties.

Thus the resistance is brought to a higher degree, but immune varieties have not yet been obtained, nor varieties with such a resistance as is necessary to solve the blight problem, although many breeding products "are promising also from the standpoint of other characters of commercial importance" (22).

PROBLEMS IN JAVA

In the temperate regions resistance is sought to Phytophthora attacks of the leaves, stems, and tubers. Tuber dry rot caused by *Phytophthora* is rather common in these potato-growing centers. This is not the case in Java; here very seldom tubers are found invaded by this fungus. It is also not known whether the same races of *Phytophthora infestans* prevailing in other countries occur in Java. The fact that certain potato varieties have a different reaction to the blight fungus in Java and in Europe shows that the aggressiveness in Java is different from that in Europe. The occurrence in Java of a new biologically specialized race or races since 1936 is not impossible, because Reddick (16) believed that *Phytophthora infestans* is

"moderately plastic." In Europe the potato variety Bevelander is more resistant to blight than Eigenheimer; but in Java the reverse is true. Such is also the case with Alpha and Robijn varieties which in Java are more susceptible than in Europe. In Java resistance to wilt disease is even more desirable than resistance to *Phytophthora* since no practical control methods for wilt are known. This resistance to wilt does not correlate with the resistance to *Phytophthora*. Varieties with some resistance, or perhaps less susceptibility, to *Bacterium solanacearum* are Arran Commander, Arran Consul, Botergele, Catriona, Gladstone, Golden Wonder, Green Mountain, Jubel, Kentang Djawa, Koninkjes, Paul Krüger, Roode Star, Sutton Flourball, Woeloeng, and M. 19; they are more resistant than Bevelander. Kentang Djawa and Woeloeng are local varieties found only in remote districts. M. 19 is a newly imported variety not yet identified (when imported the name was not known). Also, Eddins (4) found Green Mountain to be one of the more resistant varieties. Many of the varieties mentioned have been found in Java to be more susceptible to *Phytophthora* than Bevelander (see table 1). The following potato varieties received from Eddins in California have been observed in California as well as in Java to be fairly resistant to blight, but they are susceptible to *B. solanacearum* in Java: 0–55 (Chippewa × Katahdin), 1173–32, 1173–72, 1173–83, 1173–92, and 1173–136 (Spaulding Rose × Katahdin).

TABLE 1.—*The reactions of potato varieties to infection by Phytophthora infestans in relation to the reaction of the variety Bevelander*

Variety	Disease reaction[a]	Variety	Disease reaction[a]
Ally	+ 0.4	Sutton Flourball	− 0.2
Abundance	+ 1.2	Sutton Ninetyfold	+ 0.2
Americana	+ 2.2	Sutton White City	+ 0.2
Alpha	+ 0.9	Thorbecke	− 0.1
Australia 981	+ 0.6	Triumph	+ 0.3
Australia 982	+ 0.4	Up to date	+ 3.0
		Zonda di Berlino	+ 1.8
Arran Commander	− 1.0		
Arran Comrade	+ 1.4	172—18[b]	− 0.4
Arran Pilot	+ 1.5	174—29	− 0.5
Arran Crest	+ 0.4	175—37	− 1.1
Arran Cairn	+ 0.3	175—47	− 1.1
Arran Chief	− 0.3	175—52	− 0.9
Arran Consul	− 0.5	175—55	− 1.2
Arran Peak	+ 1.4	1109—8596	− 0.2
Arran Banner	+ 1.7		
		M. 8[c]	− 0.3
Bintje	+ 2.2	M. 9	0.0
Bine fantina di bhiaggia	+ 2.2	M. 10	+ 0.3
Basilicata	+ 2.2	M. 13	0.0
British Queen	+ 2.2	M. 14	+ 0.4
Duke of Kent	− 0.8	M. 18	+ 0.1
Duke of York	+ 1.4	M. 19	− 0.1
Di Vernon	+ 2.3	M. 24	+ 0.4
Donkere Roode Star	+ 0.8	M. 27	+ 0.1
Doon Star	− 0.5	M. 28	− 0.2
Donnontar Castle	+ 0.9	M. 30	+ 0.2
Eigenheimer	− 0.2	M. 32	+ 0.1
		M. 33	+ 0.1

<div align="center">TABLE 1—(Continued)</div>

Variety	Disease reaction[a]	Variety	Disease reaction[a]
Epiquire	+0.9	M. 38	−0.1
Eclipse	+1.6	M. 47	−0.2
Express	+1.6	M. 47/77	−0.2
Ella	+1.9	M. 50	−0.3
Energie	+1.1	M. 56	−0.1
Frühmölle	+0.7	M. 58	−0.5
Fransehen	+2.9	M. 60	−0.2
		M. 67	−0.1
Furore	−0.4	M. 71	−0.4
Glasgow Favorite	+0.2	M. 73	−0.3
Gladstone	−0.2		
Golden Wonder	+0.4	M. 77	−0.2
Geeltjes	+3.5	M. 139	+1.5
Hellena	−0.5	M. 164	0.0
Industrie	−0.8	M. 165	+0.4
		M. 167	+0.4
Inv. Favorite	+0.1	M. 168	−0.6
Jubel	+0.1	M. 170	−0.2
King Edward	+0.8	M. 173	−0.1
King George	+0.2	M. 175	+0.3
Limosa	+3.4	M. 177/147	−0.4
Mathilde	−0.3	M. 179	+0.5
Majestic	+1.5	M. 180	+0.3
N. Zeeland	−0.3	M. 181	+0.6
Noordeling	−0.6	M. 182	+0.1
Nationaal	−0.2	M. 182b	+1.1
Populair	−0.5	M. 183	+0.5
Pisana	+0.5	M. 184	+0.6
Parnassia	−0.6	M. 190	−0.2
Robijn	+0.2	M. 192	0.0
Red King	+2.2		
Red Skin	+0.2	1173—32[d]	−1.3
Record	−0.2	1173—72	−0.3
		1173—83	−0.1
S. Castle	+1.2	1173—92	−0.3
S. Coprina	0.0	1173—136	−0.3
S. Bellahouston	+0.2	O55	−0.2

[a] A plus rating indicates that the variety was more susceptible than Bevelander; a minus rating that it was more resistant.

[b] Hybrids from Russia, progeny of backcrosses *Solanum demissum* × *S. tuberosum*.

[c] M numbers are newly imported, not yet identified varieties.

[d] Crosses of *Solanum tuberosum* × *S. tuberosum* received from California.

Therefore these two diseases present different problems; it is desirable to have potatoes immune from both, but this would complicate the matter much more. The main problem for the temperate zones is the immunity from *Phytophthora;* in Java we must seek first the immunity from wilt disease.

<div align="center">METHODS OF TESTING</div>

The testing for resistance against *Phytophthora infestans* is done in a field where the conditions for blight epidemics are very favorable during a large part of the year, the air humidity being high.

The varieties to be tested are planted in rows separated by rows of the

variety Bevelander. The severity of attack is indicated by numbers ranging from 0 to 6. The inspection for disease rating is made 20, 30, 45, and 60 days after planting; the second and third ratings, being the most important, are valued twice as much as the others (6). The average of these ratings is compared with that of the variety Bevelander; in this way several varieties of *Solanum tuberosum* and of *S. demissum* hybrids received from Russia have been listed (Table 1). Plants were not sprayed with Bordeaux mixture.

Resistance to *Bacterium solanacearum* is tested at another place on severely infested soil, where 20 tubers of each variety are planted in rows. The tubers of the different varieties to be tested and those of the standard variety Bevelander are alternated at random. The main test for the market varieties is the yield of tubers as it is related to tuber rot during storage. The test for the wild varieties and for the hybrids has for its base the longevity of the plants. When more than 50 per cent of the planted variety remains healthy during at least 2 months, this variety is to be regarded as resistant, because during this time from 80 to 100 per cent of the variety Bevelander generally has become diseased. This test is repeated at least twice owing to the variability of the occurrence of wilt in the experimental plants. During the rainy seasons many more diseased plants generally are to be found than during the dry monsoon. The average number during a whole year indicates the grade of resistance. The standard varieties of *Solanum tuberosum* usually had more than 50 per cent wilt, and often 80–100 per cent wilt; and they are to be regarded as susceptible. Spraying with Bordeaux mixture is done regularly.

RESULTS

Besides the resistance of potato varieties to *Phytophthora* and wilt diseases, the resistance of different wild species of *Solanum* has been studied. Through the kindness of several foreign institutes the Institute for Plant-diseases now has available several wild species. Most of them are blossoming well under tropical conditions. The reverse is true for most of the varieties of *S. tuberosum*. Thus it has been necessary that part of the hybridization work be done in a temperate climate, in the Netherlands; this was the case with most of the crosses between wild species and *S. tuberosum*. The backcrosses with *S. tuberosum* as well as the reciprocal crosses have been done in Java. Varieties that blossom rather well in Java are: Americana, Arran Commander, Arran Cairn, Bevelander, Djawa, Duke of Kent, Eigenheimer, Industrie, Jubel, Pizona, Populair, Robijn, Sutton Flourball, Thorbecke, and Triumph.

The progenies of crosses with the following species of *Solanum* have been tested for their disease resistance: *S. andigenum, S. antipoviczii, S. caldasii, S. chacoense,* and *S. demissum.*

It is known that some of these potato species comprise several races. Schick and Schaper (see 19) showed the differences among several races of

TABLE 2.—*The resistance to Phytophthora infestans in 4 wild species of Solanum and in F₁ hybrids obtained when the species were crossed with S. tuberosum*

Parental wild species of Solanum	F₁ hybrids[a] with Solanum tuberosum	Resistance to Phytophthora in field tests	
		Wild species	Hybrids
	Number	Per cent	Per cent
S. andigenum	82	80	29
S. antipoviczii	6	100	33
S. caldasii	33	90	39
S. demissum	500	100	33

[a] These hybrids are F₁ progenies of hybridization with the wild species as female parent.

Solanum demissum in their resistance to four races of *Phytophthora infestans.* *S. andigenum* is also very heterogeneous (7, 19).

The wild species used in the hybridization work of the Institute for Plant-diseases proved, according to our testing experiments, very resistant to *Phytophthora* disease and also to wilt. An exception to this is *Solanum demissum;* the several races of the species available at the Institute were immune from *Phytophthora* but were not resistant to *Bacterium solanacearum.* The numbers for their percentages of resistance (Tables 2 and 3) were fixed after yearly experiments during five years.

Bukasov (3), mentioning the data concerning hybridization experiences of different institutes, reported the results of E. Schmidt, who found that in the F₁ hybrids between *Solanum demissum* and *S. tuberosum* there was 100 per cent resistance, even to the especially virulent Streckenthin race of *Phytophthora infestans,* but that the resistance was less in the later hybrids secured from backcrosses to *S. tuberosum.* Reddick (15) recorded a case in which 50 per cent of the double backcross plants of *S. demissum* proved to be immune from blight under North American conditions. Our own data for the F₁ hybrids of the wild *Solanum*-species × *S. tuberosum* are in table 2.

Table 3 gives the data on resistance to *Bacterium solanacearum* in the same F₁ progenies and in progenies of further backcrosses with *Solanum tuberosum* and of reciprocal crosses.

TABLE 3.—*The resistance to Bacterium solanacearum in 5 wild species of Solanum and in hybrids obtained from crosses between the wild species and S. tuberosum*

Wild species of Solanum	Resistance of the wild species	Resistance of the hybrids from crosses		
		Wild species × S. tuberosum	(Wild species × S. tuberosum) × S. tuberosum	S. tuberosum × (wild species × S. tuberosum)
	Per cent	Per cent[a]	Per cent[a]	Per cent[a]
S. andigenum	80	31 (153)	58 (24)	
S. antipoviczii	80	24 (25)	57 (7)	
S. caldasii	70	33 (55)	54 (222)	53 (101)
S. chacoense	70	36 (25)	50 (28)	43 (77)
S. demissum	40	18 (1369)	53 (65)	54 (101)

[a] The number in parentheses is the number of hybrid varieties tested.

The testing for resistance to *Phytophthora infestans* has not yet proceeded so far as that for wilt disease owing to the fact that every hybrid variety yields only a limited number of tubers, which first are used in the experiments concerning wilt resistance.

The F_1 progeny have a diminished resistance when compared with the original wild parent. According to the experience of other institutes this is to be expected regarding the resistance to *Phytophthora*, but it was not known for *Bacterium solanacearum*. Promising are the backcrosses with *Solanum tuberosum* and also the reciprocal crosses since they do not show further diminution of resistance. There is even an increase of resistance when these progenies are compared with the F_1 progenies. This can probably be ascribed to genetic factors, since the external conditions for wilt disease were favorable. This will be tested further during the next several years. Several of the resistant progenies, especially those of the crosses *S. tuberosum* × (wild species × *S. tuberosum*), yield well-formed tubers with rather good taste and flat eyes. The skin colors are different, varying from white to rose and dark purple, the flesh varying from white to yellow.

Reddick (15) came to the conclusion with his *Solanum demissum* crosses: "If an immune first-generation plant is backcrossed with pollen from a cultivated variety the progeny is heterogeneous." We obtained the same results with regard to the resistance to wilt with all the crosses. One backcross can yield highly resistant and immune hybrids, the next backcross susceptible ones.

From the data given, our work program has proved to be safe; still starting from resistant hybrids we shall make further backcrosses with cultivated varieties until plants will be obtained with tubers of commercial size and with high resistance, in the first place, to *Bacterium solanacearum*. Many of the successive progenies will have mostly wild features, as we found in former crosses, but some will approximate the desired characters. It is of great importance to work with large numbers.

INSTITUUT VOOR PLANTENZIEKTEN,
 BUITENZORG, JAVA.

LITERATURE CITED

1. BECKER, J. A., P. FROEHLICH, S. W. MENDUM, L. D. HOWELL, F. J. HOSKING, and G. W. SPRAGUE. "Agricultural Statistics." U. S. Dept. of Agr. Yearbook. **1935**: 345–746. 1935.
2. BUIJZE, P. "Slijmziekte, de plaag der aardappelcultuur." De Indische Boerderij **7**: 1–2. 1929.
3. BUKASOV, S. M. The problems of potato breeding. Amer. Potato Jour. **13**: 235–252. 1936.
4. EDDINS, A. H. Brown rot of Irish potatoes and its control. Fla. Agr. Exp. Sta. Bul. 299. 1936.
5. EEK, TH. VAN. De huidige stand van het aardappelonderzoek in Ned. Indië. Verslag 27ste verg. V.V.P.P. 76–93. 1939.
6. ————, and T. H. THUNG. Resultaten van het aardappelonderzoek. In manuscript form.
7. EMME, H. and M. VESELOVSKAJA. Zur Frage der Bedeutung der Formen von *S. andigenum* Juz. et Buk. für die Züchtung. Der Züchter **7**: 25–29. 1935.
8. GOOT, P. VAN DER. Overzicht der voornaamste ziekten van het aardappelgewas op Java. Inst. v. Plantenziekten. Bul. 18. 1924.

9. Muller, H. R. A. De aardappelsituatie op Java als gevolg van het optreden van eenige nieuwe ziekten. Landbouw. 13: 285–313. 1937.
10. Müller, K. O. Über den augenblicklichen Stand unserer Kenntnisse zur biologischen Spezialisierung des Krautfäuleerregers der Kartoffel (*Phytophthora infestans*). Der Züchter 7: 5–12. 1935.
11. Nakata, K. Principles for controlling tobacco wilts. Jour. Sci. Agr. Soc. No. 298. 1927.
12. Paravicini, E. M. M. De aardappelcultuur in Ned. Indië. Teysmania 5–6: 191–240. 1922.
13. Poel, J. van der. Overzicht van de thans verkregen resultaten van het onderzoek naar den invloed van verschillende mestatoffen op de slijmziekte. Med. Deli Proefstă. Tweede Serie No. 159. 1938.
14. Reddick, D. Blight-resistant potatoes. Phytopath. 18: 483–502. 1928.
15. —————. Elimination of potato late blight from North America. Phytopath. 24: 555–557. 1934.
16. —————. Problems in breeding for disease resistance. Chronica Botanica 6: 73–77. 1940.
17. Roemer, Th., W. H. Fuchs, and K. Isenbeck. Die Züchtung resistenter Rassen der Kulturpflanzen. 427 pp. Berlin. 1938.
18. Salaman, R. N. The present state and future development of potato breeding. Indian Jour. Agr. Science 8: 119–129. 1938.
19. Schick, R. Untersuchungen über den Wert des *Solanum andigenum* für die Kartoffel-züchtung. Der Züchter 6: 273–280. 1934.
20. Schopruijs, H. J. De aardappelsituatie in Ned. Indië. Rapport Dept. E. Z. 1941.
21. Sidorov, F. F. Züchtung *Phytophthora* widerstandsfähiger Kartoffelsorten. Phytopath. 27: 211–241. 1937.
22. Stevenson, F. J., and C. F. Clark. Breeding and genetics in potato improvement. U. S. Dept. of Agr. Yearbook 1937: 405–444. 1937.
23. —————, E. S. Schultz, C. F. Clark, N. P. Raleigh, L. C. Cash, and R. Bonde. Breeding for resistance to late blight in the potato. Amer. Potato Jour. 13: 205–218. 1936.
24. Thung, T. H. De huidige stand van het Phytophthora-vraagstuk in de Vorsten-landen. Med. Proefstă. Vorstenl. Tabak No. 74. 1932.

LEAF BLIGHT OF PINK CALLA CAUSED BY PHYTOPHTHORA ERYTHROSEPTICA

C . M . T O M P K I N S   A N D   C . M . T U C K E R [1]

INTRODUCTION

A serious leaf blight of the Superba variety of pink calla (*Zantedeschia rehmannii* Engler) was first observed during the summer of 1938 in field plantings in the Monterey Bay region of California. The disease has also been prevalent in subsequent years, causing appreciable loss wherever found. The symptoms of this disease, the causal organism, host range, and suggestions for control are discussed in this paper.

REVIEW OF LITERATURE

In 1912, Pethybridge (5) described a new tuber rot of the potato (*Solanum tuberosum* L.) which was first observed in 1909 at Clifden, County Galway, Ireland. A more detailed account (6) followed in 1913, in which he suggested the common name of pink rot, descriptive of invaded tissues which, on exposure to the air, turned pink but later became dark-brown or black. In the same paper, he proposed that the causal fungus be named *Phytophthora erythroseptica* Pethyb. Still later, Pethybridge (7) mentioned wilting of the foliage and stalks above ground, rotting of the stalks below the ground level, and partial decay of some of the roots and rhizomes, in addition to tuber rot, as conspicuous phases of the disease. Therefore, he suggested that the common name "pink-rot" be changed to "pink-rot-wilt." Two decades later, Cairns and Muskett (3) corroborated the facts as presented by Pethybridge (5, 6, and 7), emphasizing that normally the disease is of importance only as a tuber disease. High humidity and poor aeration were also shown to cause heavy loss in affected crops in storage. According to Cairns and Muskett (3) and Tucker (10), the disease has been reported from Scotland, England, Wales, Holland, Bulgaria, and Java, Netherlands East Indies. In 1944 *P. erythroseptica* was isolated from tubers in Idaho by E. C. Blodgett, and in 1945 isolates of the species from tubers in Nebraska were received from R. W. Goss. In both instances the species was identified by one of the writers. Recently, White (13) reported the disease in Tasmania.

Westerdijk and van Luijk (12) reported a root rot of *Atropa belladonna* L., caused by *Phytophthora erythroseptica*, in Holland in 1920. Later, in 1926, Alcock (1) observed that this fungus attacked the base of the stem of this host in early summer in England. Above the point of attack, severe wilting of the foliage occurred. The fungus also caused damping-off of

belladonna seedlings. Alcock suggested the name *P. erythroseptica* var. *atropae*, but Tucker (9) considers the varietal name unwarranted.
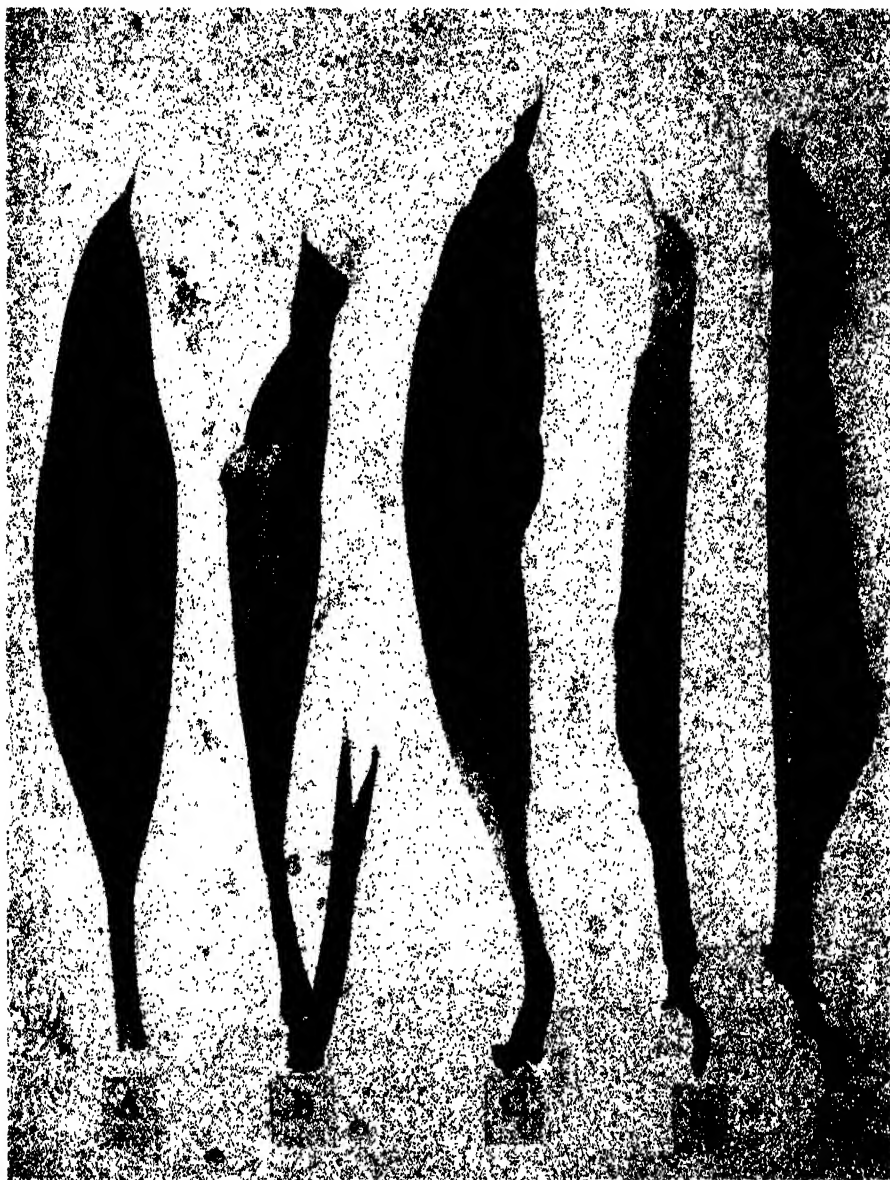
FIG. 1. Leaves of pink calla, variety Superba, grown from flowering-size corms. A. Healthy leaf from control plant. B, C, D, E. Four leaves having a brownish-black discoloration of the lower part of the petioles, with chlorosis and distortion of the leaf laminae, 22 days after inoculation of the plant with *Phytophthora erythroseptica*.

In 1938, Buddin (2) described a root rot, shoot rot, and shanking of tulip (*Tulipa gesneriana* L.), caused by *Phytophthora cryptogea* Pethybr. and

Laff. and *P. erythroseptica*, which had caused great damage in greenhouse-forced crops.

SYMPTOMS OF THE DISEASE

With the onset of the disease, the clusters of narrow, green leaves of pink calla plants, grown either from seeds or corms, suddenly show a general

FIG. 2. Pink calla plants, variety Superba, grown from flowering-size corms. A. Healthy control plant. B, C, D. Diseased plants, showing progressive symptoms, from mild to severe, 22 days after inoculation with *Phytophthora erythroseptica*.

chlorosis and are less turgid than healthy specimens. Infected leaves soon turn yellow and become distorted, with a tendency for the edges to curl upward. Simultaneously, for a distance of several centimeters above and below the surface of the soil, a wet, odorless, brownish-black discoloration of the petioles is noticeable (Fig. 1). At first, this is firm but soon turns soft and becomes mushy. With the rapid destruction of the supporting tissues in the petioles, the leaves collapse, singly or in groups, until all in the cluster are prostrate. This occurs within a few hours, and death of the foliage ensues within a day or two (Fig. 2). Even in the most severe cases, no infection of the roots or corms of blighted plants has been observed.

Apparently the disease is favored by cool, foggy weather, excessive irrigation, and heavy, poorly drained soils.

THE CAUSAL FUNGUS, PHYTOPHTHORA ERYTHROSEPTICA

Isolations made from diseased pink calla leaves on malt-extract agar have consistently yielded a fungus which has been identified as *Phytophthora erythroseptica* Pethybr.

Cultures on the usual media incubated at room temperatures seldom develop fruiting bodies with the exception of occasional hyaline, spheroidal structures regarded as poorly differentiated chlamydospores. Cultures on oatmeal agar incubated one month at 8° C. develop sporangia sparingly, and oogonia, oospores, and antheridia very abundantly. Morphologic studies were made on 2 isolates which proved indistinguishable.

The sporangia are hyaline, ellipsoidal to obpyriform, terminal on long sporangiophores not differentiated from the vegetative hyphae or on short lateral branches, nonpapillate, but often provided with a lenticular hyaline plug at the slightly flattened apex; 29–39 × 15–26 μ, mean 34.6 × 20.7 μ. Germination occurs by germ tubes or zoospore formation; in the latter case, evacuation of the sporangium is frequently followed by renewed growth of the sporangiophore through the base of the sporangium, and the development of another sporangium within or beyond the first.

Sexual reproduction occurs in great profusion. The oogonia are hyaline, spheroidal, tapering abruptly to a slender stalk completely encompassed by the persistent, amphigynous antheridium; 27–44 μ in diameter, mean 35.8 μ. Oospores single, spheroidal, cream to straw-color, wall thick (3–4 μ), contents densely granular, occurrence of reserve globules irregular and varying with age of spores; germination was not observed; 20–36 μ in diameter, mean 29.4 μ. Antheridia roughly spheroidal, but frequently irregular and distorted, sometimes with one or two finger-like protuberances, amphigynous, intimately and permanently enclosing the slender oogonial stalk; 12–19 μ in diameter.

The oogonia and oospores correspond closely in size to the description by Pethybridge (6), but are slightly smaller than those reported by Tucker (9); their abundant development in the calla isolates may have been a factor in determining size. Comparison of the calla isolates with the Idaho and

Nebraska isolates from potato tubers, both of which produce oogonia in large numbers, failed to reveal significant differences in morphology. The similarity of the isolates in temperature-growth relations provides additional evidence of their relationship.

The calla isolates may be distinguished from the potato isolates of *Phytophthora erythroseptica* by their failure to invade and cause a pink rot of potato tubers. Tubers inoculated at incisions with the potato isolates were almost completely invaded after one week; those inoculated with the calla isolates did not develop symptoms of invasion. Tucker (9), using isolates of *P. cactorum* (L. and C.) Schroet., *P. palmivora* Butl., and *P. parasitica* Dast. from numerous hosts, showed that isolates of the 3 species from different origins may vary widely in pathogenicity. Alcock (1) reported that an isolate of *P. erythroseptica* from *Atropa belladonna* caused a rot of potato tubers which did not develop the pink color characteristic of infections by potato isolates. The failure of the calla isolate to infect potato tubers is evidence of the occurrence of physiologic specialization in *P. erythroseptica*, but does not provide a valid basis for its consideration as the type of a new species or variety.

Isolates of the fungus proved pathogenic to healthy pink calla plants grown from healthy corms. Inoculum was prepared by growing the fungus on sterilized cracked wheat in 8-inch test tubes. When ready for use, this was added to autoclaved soil contained in 6-inch pots in such a manner as to avoid injuring the root system of the young plants. Sterile cracked wheat was added to the pots serving as controls. The air temperature in the greenhouse in which the tests were conducted, ranged from 13° to 19° C. All pots were heavily watered each day in order to keep the soil very moist and thus provide optimum conditions for infection. The incubation period ranged from 22 to 29 days, but all leaves of infected plants died within a day or two after the foliage had collapsed (Figs. 1 and 2). Of 30 plants inoculated, none escaped infection, while the 30 control plants remained healthy. The fungus was reisolated from several petioles of each infected plant, and it proved to be identical with the original isolate. The reisolates proved highly pathogenic. In these tests, the infected plants exhibited symptoms identical with those of naturally infected plants. In all infection tests, the corms which had produced blighted leaves remained firm and healthy.

The relation of temperature to growth of the mycelium was studied. The culture tubes (2.1 by 20 cm.) used, and the procedure followed, were those previously described by Tompkins and Gardner (8). The medium used was malt-extract agar, pH 7.0. Inoculated tubes were kept at room temperature for 48 hours. Then 3 tubes of the isolate were placed in a horizontal position in controlled temperature chambers at intervals of 3° from 4° to 40° C. The cultures were incubated for 96 hours. The cardinal temperatures were determined on the extent of mycelial growth in the culture tubes.

The minimum temperature for growth of the pink-calla isolate of *Phy-*

tophthora erythroseptica was approximately 13° C., the optimum 25°, and the maximum 28°. Using a strain of the fungus isolated from potato in Holland, Tucker (9) observed that it grew only slightly on corn-meal agar at 15°, profusely at 27.5°, and failed to develop at 30°. Thus, the temperature relations of the pink-calla isolate are in close agreement with those of the potato isolate. In their studies, Cairns and Muskett (4) showed that the minimum, optimum, and maximum growth temperatures of a potato isolate were 5°, 25°, and 31° C.

EXPERIMENTAL HOST RANGE

A survey of the literature indicates that the host range of *Phytophthora erythroseptica* is extremely limited. In laboratory tests, Pethybridge (6) obtained artificial infection of aseptically-prepared blocks of living tissue of mangel (*Beta vulgaris* L.), white turnip (*Brassica rapa* L.), and Swede (*B. campestris* L. var. *napo-brassica* DC.), but failed with carrot (*Daucus carota* L.) and parsnip (*Pastinaca sativa* L.). Swedes and white turnip roots were successfully infected through wound-inoculation; the fungus was highly pathogenic to turnip. Tucker (9) found the species virulently pathogenic to wounded green fruits of tomato (*Lycopersicum esculentum* Mill.), and weakly pathogenic to wounded apple fruits (*Pyrus malus* L.).

Limited studies on the host range of the pink-calla isolate of *Phytophthora erythroseptica* were conducted in the greenhouse and laboratory. In the former, using the method of inoculation described for the tests on pathogenicity, 6 yellow calla plants (*Zantedeschia elliottiana* Engler) of 8 inoculated were infected after 28 days. The foliage symptoms were comparable to those obtaining on infected pink-calla plants. No infection was obtained on plants of white calla (*Z. aethiopica* Spreng.),[2] Fiery Blood Red annual stock (*Matthiola incana* R. Br. var. *annua* Voss), tomato (*Lycopersicum esculentum* Mill.), or China aster (*Callistephus chinensis* Nees). In the laboratory, no infection was obtained by placing agar inoculum on the surface of healthy white calla rhizomes, on corms of pink and yellow calla, and tubers of tuberous-rooted Begonia (*Begonia tuberhybrida* Voss) and White Rose potato.

DISCUSSION

Although infection of pink calla plants by *Phytophthora erythroseptica* is chiefly characterized by wilting and subsequent death of the foliage, apparently the fungus is unable to parasitize the roots and corms. However, additional loss is reflected in the condition of the mother corms which were used originally as planting stock. As a result of foliage failure, especially if it occurs early in the season, there is little or no increase in the size of the mother corms, and no new offsets or cormels are formed. After harvesting and curing, the mother corms from infected plants may again be used as planting stock, but undoubtedly their vigor has been reduced.

[2] After completion of this manuscript, *Phytophthora erythroseptica* was isolated at harvest time from diseased white calla rhizomes grown on the San Francisco Peninsula. A full report on this disease will be published in the near future.

Avoidance of this disease can best be obtained by careful selection of clean, well-drained soils and by the judicious use of irrigation water. These factors are of paramount importance in averting losses. Pethybridge (6, 7), Cairns and Muskett (3, 4), and Van Haeringhen (11) made similar recommendations relative to the control of pink rot of potato.

SUMMARY

A leaf blight of pink calla is prevalent in the Monterey Bay region of California.

Symptoms of the disease consist of rapid wilting, yellowing, and collapse of the leaves, with blackening of the petioles at and slightly above and below the soil level. Death of the foliage occurs in a few days.

The causal organism has been identified as *Phytophthora erythroseptica* Pethybr. on the basis of morphologic characters and temperature-growth relations. It differs from isolates from potato in pathogenicity, failing to infect and cause a pink rot of potato tubers.

In the greenhouse, infection was obtained by adding the fungus to the wet, autoclaved soil of potted plants grown from healthy corms. The incubation period averaged 25½ days.

The pink-calla isolate of the fungus was pathogenic to yellow-calla potted plants.

The minimum temperature for mycelial growth was 13° C., the optimum 25°, and the maximum 28°.

The disease is favored by wet, poorly drained soil and by cool, foggy weather. Crop rotation and sanitation are suggested as a method of control.

This is apparently the first record of *Phytophthora erythroseptica* as a pathogen on pink calla.

DIVISION OF PLANT PATHOLOGY,

    CALIFORNIA AGRICULTURAL EXPERIMENT STATION,

      BERKELEY, CALIFORNIA,

    AND

DEPARTMENT OF BOTANY,

    MISSOURI AGRICULTURAL EXPERIMENT STATION,

      COLUMBIA, MISSOURI

LITERATURE CITED

1. ALCOCK, N. L. A preliminary note on a Phytophthora on *Atropa belladonna*. Pharm. Jour. & Pharmacist 116(3254): 232. 1926.
2. BUDDIN, WALTER. Root rot, shoot rot and shanking of tulip caused by *Phytophthora cryptogea* Pethybr. and Laff. and *P. erythroseptica* Pethybr. Ann. Appl. Biol. 25: 705–729. 1938.
3. CAIRNS, H., and A. E. MUSKETT. Pink rot of the potato. Ann. Appl. Biol. 20: 381–403. 1933.
4. ——————, and ——————. *Phytophthora erythroseptica* Pethybr. in relation to its environment. Ann. Appl. Biol. 26: 470–480. 1939.
5. PETHYBRIDGE, GEORGE H. Investigations on potato diseases. Third report. Jour. Dept. Agr. and Tech. Instr. Ireland 12: 334–359. 1912.
6. ——————. On the rotting of potato tubers by a new species of Phytophthora, having a method of sexual reproduction hitherto undescribed. Royal Dublin Soc. Sci. Proc. 13(n.s.): 529–565. 1913.

7. ——————. Further observations on *Phytophthora erythroseptica*, Pethyb., and on the disease produced by it in the potato plant. Royal Dublin Soc. Sci. Proc. **14**(n.s.): 179–198. 1914.

8. TOMPKINS, C. M., and M. W. GARDNER. Relation of temperature to infection of bean and cowpea seedlings by *Rhizoctonia bataticola*. Hilgardia **9**: 219–230. 1935.

9. TUCKER, C. M. Taxonomy of the genus Phytophthora deBary. Missouri Agr. Exp. Sta. Res. Bul. 153. 1931.

10. ——————. The distribution of the genus Phytophthora. Missouri Agr. Exp. Sta. Res. Bul. 184. 1933.

11. VAN HAERINGHEN, G. H. Eenige waarnemingen in de praktijk over *Phytophthora erythroseptica*. Tijdschr. Plantenziekten **45**: 247–256. 1938.

12. WESTERDLIK, J., and A. VAN LUIJK. *Phytophthora erythroseptica* Peth. als parasit von *Atropa belladonna*. Meded. Phytopath. Lab. "Willie Commelin Scholten" **4**: 31–32. 1920.

13. WHITE, N. H. Plant disease survey of Tasmania for the three year period 1943, 1944, 1945. Tasmanian Dept. Agr. (Mimeographed), 30 pp. 1946.

ACCELERATION AND RETARDATION OF GERMINATION OF SOME VEGETABLE SEEDS RESULTING FROM TREATMENT WITH COPPER FUNGICIDES[1]

AUBREY ALFRED FOSTER

In recent years the use of fungicides to protect vegetable seeds and seedlings from attack by microorganisms has become an economic necessity for the commercial production of many crops. The literature of plant pathology has reported enough information to indicate that the value of treatments to seed varies with experimental conditions. A chemical which works well on one kind of seed may be useless or dangerous on another. The fungicidal power of a compound against one organism is often no criterion of its strength against a different pathogen. Many other factors such as soil pH, temperature, water, age of seed, and light intensity complicate the picture further. The purpose of this study is to explain some differences observed in the effect of two fungicides containing copper on seven kinds of vegetable seeds.

LITERATURE REVIEW

Previous workers have reported injury by fungicides containing copper to seeds of peas (3, 10, 11, 12, 13, 15, 24, 25, 26, 37, 41), cucumbers (32), and crucifers (7, 8, 23). There are other crops, however, on which consistently beneficial results of fungicides containing copper have been obtained. Beets (14, 19, 24, 25), spinach (8, 9, 16, 36), eggplant, pepper and tomato (33, 34) are examples. The authors who report these results make no claim of benefit to the seeds, but attribute all of the success to protection from attacks by fungi.

The evidence presented here shows that the effect of copper compounds on vegetable seeds may be either to accelerate or retard germination. The nature of the enzyme systems responsible for respiration in the seeds determines whether the action of copper is beneficial or injurious. One enzyme system present in barley (30, 31), oats (4), cucumbers (2), and peas (17, 21) requires the presence of sulfhydryl groups in one or more of its enzymes. Enzymes of this type are inhibited by copper (1, 20, 22, 29, 35, 39). A different enzyme system found in potatoes (38), beets (42), spinach (5), and mushrooms (27) contains the enzyme tyrosinase, which requires the presence of copper ions for its activity. Plants of this latter type might be benefited rather than injured by moderate amounts of copper.

MATERIALS AND METHODS

The seven kinds of vegetable seeds[2] listed in table 1 were immersed in 1.5 per cent copper sulfate solution at room temperature for one hour. A

duplicate set was revolved in Erlenmeyer flasks for ten minutes in the presence of the quantities of cuprous oxide[3] listed in table 1.

Germination tests were made with lots of 60 seeds each that had been stored for varying times at controlled humidities and temperatures. Twenty from each lot were placed on moist filter paper in Petri plates in the laboratory, 20 were planted in the greenhouse in steamed soil, and 20 were planted in artificially infested greenhouse soil containing parasitic strains of *Pythium, Corticium,* and *Fusarium.*

Germination was counted daily for two weeks in the laboratory (three weeks for eggplant and pepper), and seedling emergence counted daily in the greenhouse for three weeks (four weeks for eggplant and pepper). A daily count of post-emergence damping-off was also made. The figures for emergence in infested soil represent final stands at the end of the germination period. The others are total emergence. Coefficients of velocity calculated by the Kotowski (28) method give a measure of speed of germination.

TABLE 1.—*Dosage of cuprous oxide used in treating seven kinds of vegetable seeds*

Kind	Variety	Dosage, per cent by weight
Beet	Detroit Dark Red	1.13
Cabbage	Glory of Enkhuisen	0.75
Cucumber	Arlington White Spine	0.38
Eggplant	Black Beauty	0.75
Pea	Telephone	0.38
Pepper	California Wonder	0.75
Spinach	Bloomsdale	0.75

Plantings were randomized and significance of differences determined by analysis of variance.

A second test was conducted in which ten, 50-seed lots of peas and ten, 100-seed lots of spinach were planted in steamed soil. A duplicate series was placed in Petri plates. Cuprous oxide was used at the same dosage as before and records taken in the same manner.

For studies of oxygen uptake, samples of seeds and seedlings ground in a mortar were diluted to 10 per cent by weight with a solution containing M/20 sodium succinate and M/30 phosphate buffer at pH 6.5. The suspensions were strained through a double layer of fine cheesecloth and placed in Fenn differential volumeters. Cuprous oxide at a concentration of 0.007 per cent was added to the ear of the vessel for studies of respiratory inhibition.

Imbibition by seeds or seedlings was determined by recording the difference between fresh weight and dry weight after heating to 80° C. in a vacuum oven for 24 hours.

Copper was determined by the Callan and Henderson (6) method. To a slightly ammoniacal tissue suspension 10 per cent by volume of a 0.1 per cent sodium diethyl-dithio-carbamate solution was added. Color intensity was measured in a Cenco-Shear-Sanford photelometer with a No. 1 blue filter.

[3] "Yellow cuprocide" furnished by Rohm and Haas Company, Philadelphia, Pennsylvania.

Sulfhdryl groups were determined by the nitroprusside method (18, 40). Two grams of ground tissue were taken up in 5 ml. distilled water. Five drops of 10 per cent sodium nitroprusside solution, 2 g. of solid ammonium sulfate, and 3 ml. of 10 per cent acetic acid were added and the tube was shaken. Addition of an excess (one ml.) of concentrated ammonium hydroxide caused a pink coloration in the presence of sulfhydryl groups.

EXPERIMENTAL RESULTS

The results of germination tests of seeds treated with cuprous oxide and copper sulfate are presented in table 2. The seeds can be divided into two groups based on their reaction to the fungicide in the absence of pathogens. Beet, eggplant, pepper, and spinach germination is increased or hastened in one or more cases by treatment. Cabbage, cucumber, and pea germination,

TABLE 2.—*The germination of seven kinds of vegetable seeds dusted with cuprous oxide or soaked in 1.5 per cent copper sulfate solution. (Means of 24 samples, 20 seeds each)*

Kind of seed	Treatment	Petri plates		Emergence from soil	
		Germination, per cent	Coeff. of velocity	Steamed	Infested
Beet	Copper sulfate	59	20.0	133	74
	Cuprous oxide	50	19.8	117	77
	None	37	22.4	123	49
	Least significant dif. at 19:1	10	N.S.	N.S.	16
Eggplant	Copper sulfate	56	16.0	60	36
	Cuprous oxide	59	16.1	64	41
	None	48	13.1	63	22
	L.S.D. at 19:1	N.S.	2.3	N.S.	9
Pepper	Copper sulfate	62	11.7	62	52
	Cuprous oxide	70	12.1	64	35
	None	63	10.7	61	22
	L.S.D. at 19:1	N.S.	1.2	N.S.	11
Spinach	Copper sulfate	50	17.2	65	32
	Cuprous oxide	38	16.6	67	28
	None	22	20.6	64	20
	L.S.D. at 19:1	13	N.S.	N.S.	9
Cabbage	Copper sulfate	8	47.0	29	42
	Cuprous oxide	65	35.4	60	42
	None	68	40.1	61	56
	L.S.D. at 19:1	9	N.S.	9	8
Cucumber	Copper sulfate	85[a]	59.3	78	66
	Cuprous oxide	93	50.5	85	69
	None	88	61.2	84	34
	L.S.D. at 19:1	N.S.	4.3	N.S.	12
Pea	Copper sulfate	22	24.8	64	20
	Cuprous oxide	54	24.9	82	51
	None	67	29.2	78	18
	L.S.D. at 19:1	9	1.8	7	11

[a] Sprouts blue, stunted, not geotropic.

on the other hand, is reduced or retarded in one or more cases.   The column reporting results in infested soil shows that injury to the seed may be obscured by the protective action of the chemical.   In the presence of a high concentration of active pathogens the cucumber and pea seeds were actually benefited by treatment.   Germination of these seeds was reduced so much by pathogens that the effect of chemicals on the seed itself was not obvious.

Spinach seeds were selected as representative of the type in which germination was accelerated by copper, and pea seeds as representative of the type in which germination was retarded.   Lots of 50 pea seeds and 100 spinach seeds that had been treated with cuprous oxide were germinated in Petri dishes and steamed soil in comparison with nontreated seeds.   Results are presented in table 3 and figure 1.   A significant acceleration of
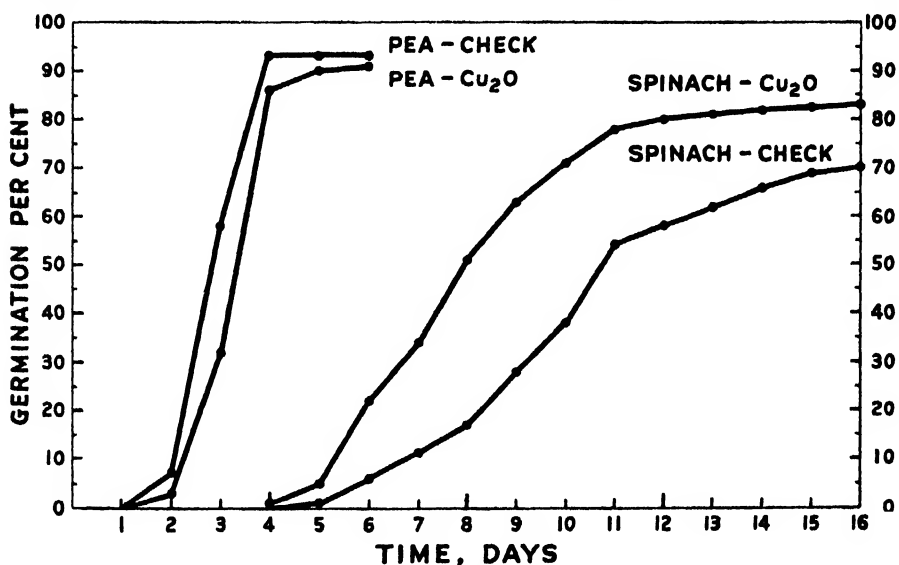
FIG. 1.   The germination of seeds of pea and spinach after treatment with cuprous oxide dust.

germination of spinach seed was observed in both Petri plates and steamed soil.   Pea germination was retarded in the Petri plates.

This evidence was considered adequate proof that the two kinds of seed responded differently to treatment with copper compounds.   The reasons for this difference remained to be demonstrated.   One possibility might be differences in the penetrability of copper ions into the seed protoplasm. Analyses of various parts of the young seedlings showed that copper had penetrated readily into treated seeds of both spinach and pea.   Thus, copper ions present in the protoplasm of pea seedlings retard development while the same ions present in spinach accelerate it.

Another possible reason for the difference in response was that the fungicide altered the permeability of the seed coat to water.   Seeds of peas and spinach were immersed for from one-half hour to 24 hours in distilled

TABLE 3.—*Effect of cuprous oxide dust on germination of pea and spinach seeds in the absence of pathogens.* (*Means of 10 samples of 50 pea and 100 spinach seeds each*)

| Kind of seed | Dosage (per cent by wt.) | Petri plate | | Steamed soil | |
		Germination (per cent)	Coeff. of velocity	Emergence (per cent)	Coeff. of velocity
Pea	0.38	90.2	27.3	90.6	17.0
	None	95.0	30.2	89.4	17.1
L.S.D. at 19:1		3.3	1.5	N.S.	N.S.
Spinach	0.75	83.2	12.3	76.2	15.1
	None	70.1	9.9	71.0	15.4
L.S.D. at 19:1		5.8	1.5	3.3	N.S.

water. No significant differences could be detected in the amount of water taken up by treated and check seeds. However, if pea seeds were placed in Petri plates for four days on filter paper moistened with distilled water, the presence of cuprous oxide retarded water uptake.

The difference in water uptake demonstrated in Petri plates had not been observed in immersion tests. It seemed probable, therefore, that the utilization of water for metabolic processes had been modified by the copper and that permeability of the seed coat was not the main factor.

A quantitative measure of metabolic activity of germinating pea seeds in the absence of light can be had by dissecting out the embryo and determining its dry weight. It is assumed that the amount of dry matter converted from endosperm to embryo is a measure of the rate of metabolism. Accordingly dry weight determinations were made on four lots of 25 dissected pea embryos from the treated seedlings and an equal number from the nontreated. Results are presented in table 4.

The significantly lower fresh weight and dry weight of embryos from treated seeds is reasonable evidence that metabolic processes responsible for conversion of dry matter from endosperm to embryo were retarded in peas by the presence of copper ions. This test was not applied to spinach seedlings because the nature of the seed makes it impossible to dissect out the endosperm.

The evidence thus far indicates that copper ions present in spinach seeds accelerate germination while the same ions in peas retard germination. Moreover, the retardation in pea seedlings is related to metabolic activities

TABLE 4.—*Effect of cuprous oxide on the dry weight and water content of pea embryos from seed germinated in Petri plates.* (*Mean of 4 samples*)

Treatment	Fresh weight, grams per 100 seeds	Dry weight, grams per 100 seeds	Water content, grams per gram of dry weight
Cuprous oxide	5.41	0.595	8.1
Check	8.61	0.891	8.7
L.S.D. at 19:1	1.16	0.020	N.S.

responsible for conversion of dry matter from endosperm to embryo. When it is considered that the energy source for all the reactions accompanying the transformation is respiration, it is logical to look next for a possible effect of copper on respiratory rate. Consequently, an investigation of the nature of enzymes used in seed respiration was made.

It has been known for some time that enzymes containing sulfhydryl groups were inactivated by copper at relatively weak concentrations. Accordingly, the nitroprusside test for sulfhydryl groups was applied to ground seeds of the seven types of vegetables used in the first tests. Positive tests were obtained with cabbage, cucumber, and pea; and negative tests with beet, eggplant, pepper, and spinach. It was thus possible to distinguish by a simple test tube method between the two groups of seeds which had been separated by germination responses.

The next question to investigate was whether copper injury to seeds of the type containing sulfhydryl groups was accompanied by destruction of the sulfhydryl groups. It was found that cucumber and pea seeds that had been treated with copper sulfate and stored ten months over calcium chloride no longer contained sulfhydryl groups. Comparable seeds that had been stored after treatment with cuprous oxide had a greatly reduced amount of sulfhydryl groups present. Nontreated seeds from the same storage maintained their sulfhydryl groups, as indicated by positive tests.

The final demonstration that copper injury to seeds was related to destruction of sulfhydryl groups was accomplished with two lots of 50 pea seeds which had been treated ten months previously with copper sulfate. These seeds would not germinate by any ordinary means and had been considered dead. When one of these lots of pea seed was placed in a Petri plate and moistened with 10 ml. of a 0.1 per cent aqueous solution of the amino acid, cysteine,[4] fifteen of the seeds germinated within two days. The water check did not germinate at all. It is obvious therefore that the inactivation of germination caused by copper can be reversed if a material rich in sulfhydryl groups is added to replace those destroyed in the seeds.

The evidence pointed to the conclusion that the effect of copper on seeds is a modification of respiratory activity. This was confirmed with ground spinach and pea seeds placed in the Fenn respirometer. Average oxygen uptake of ground spinach seed was increased by the addition of cuprous oxide, while oxygen uptake of ground pea seed was reduced. The acceleration and retardation caused by copper was thus seen to exist in ground tissue respiration in the same manner as it did in germination tests.

DISCUSSION

The data justify the separation of the vegetable seeds studied into two groups. One contains beet, eggplant, pepper, and spinach in which germination is accelerated or increased by treatment with copper sulfate or

[4] Cysteine hydrochloride, C.P.

cuprous oxide. The other contains pea, cucumber, and cabbage in which germination is retarded or decreased by the fungicides.

In making this separation it is important to realize that relative copper concentration must be considered. It is possible to kill all seeds with a sufficiently high dosage of copper. Likewise, the use of minimum quantities of copper compounds in relatively insoluble forms, and in the presence of protective colloids or antagonistic ions is safe even on seeds which are sensitive to copper. These factors make it inevitable that in the literature it is possible to find evidence contradictory to the findings of this paper.

When fungi are present, variation in pathogenicity will also complicate results. Under the conditions of the tests reported here, fungi in the infested soil caused cucumbers to damp-off badly and, therefore, treatment with copper appeared beneficial. Peas, somewhat less susceptible to damping-off, benefited with only one of the fungicides. Cabbage, however, was relatively less susceptible to the organisms present, and the injurious effect of the copper appeared even in the presence of pathogens. There are, therefore, conditions under which a grower can afford to ignore the injurious effects of chemicals because of their relative insignificance in comparison with other factors.

In classifying seeds arbitrarily into two groups there is danger of over-simplification. It is probable that many kinds of seeds contain both copper-sensitive and copper-containing enzymes. The results reported here indicate the type of enzyme predominant in the seeds studied. If copper is present, peas might be forced to respire through other systems normally unimportant. Likewise, spinach seed in the absence of copper might develop other types of enzymes. Until further work is done on this subject any interpretation must remain tentative.

The use of a test of sulfhydryl groups in determining the mode of action of a fungicide has its limitations. Any oxidizing agent will destroy sulfhydryl groups in ground seeds in a test tube. The writer used chloranil, tetramethyl-thiuram-disulfide, chlorphenol mercury, and zinc oxide. However, the copper compounds were the only ones found to destroy the sulfhydryl groups in intact seeds. Destruction of sulfhydryl groups by copper is more rapid and complete than can be explained by ordinary oxidation.

Although the presence of enzymes containing sulfhydryl groups in peas has been proved, it should be pointed out that the presence of a copper containing enzyme in spinach seed has not been conclusively proved in this work. The inference is that one is present, and the literature to date agrees with the theory.

Because of specific injury by copper to certain types of seeds, it may be well to reconsider the interpretations placed on standard seed treatment research. Frequently a group of several compounds is used in comparison with a check, and the compound which gives the greatest number of healthy seedlings is considered the most potent fungicide. It is obvious that the kind of seed used will determine the relative rating of the chemicals.

Chloranil, for instance, is outstandingly superior to cuprous oxide for treatment of peas or beans. If the two fungicides are compared on beet or spinach seed, however, their relative superiority is reversed.

SUMMARY

Seeds of beet, cabbage, cucumber, eggplant, pea, pepper, and spinach were germinated in Petri plates, steamed soil, and infested soil after seed treatment with copper sulfate and cuprous oxide.

The fungicides reduce or retard germination of cabbage, cucumber, and pea but increase or accelerate germination of beet, eggplant, pepper, and spinach.

Nitroprusside tests show that seeds of the three kinds susceptible to copper injury contain sulfhydryl groups, and those of the four kinds which are tolerant to copper do not.

Copper sulfate and cuprous oxide penetrate pea and cucumber seeds and destroy sulfhydryl groups normally present in them.

Moistening with cysteine solution partially restores the germinative capacity of pea seeds that have been injured by copper sulfate.

Cuprous oxide injury to pea seed is observed in a reduction of dry and fresh weights of embryos from seeds germinated in Petri plates.

Manometric measurements of oxygen uptake of ground seeds and seedlings show that suspensions of cuprous oxide reduce respiration of peas but not spinach.

CENTRAL FLORIDA EXPERIMENT STATION,

SANFORD, FLORIDA

LITERATURE CITED

1. AKESON, A. Cystin- und Methioninbestimmungen bei Cytochrome. Acta Physiol. Scand. 4: 362–364. 1942. (Abstr. in Chem. Zentr. 1, 1943: 2500. 1943.)
2. BASU, K. P., and J. N. KARKUN. Succinic acid dehydrogenase from cucumber seeds. Jour. Indian Chem. Soc. 20: 277–281. 1943.
3. BAYLIS, G. T. S., R. S. DESHPANDE, and J. F. STOREY. Effect of seed treatment on emergence of peas. Ann. Appl. Biol. 30: 19–26. 1943.
4. BERGER, J., and G. S. AVERY, JR. Glutamic and isocitric acid dehydrogenases in the Avena coleoptile and the effect of auxins on these enzymes. Amer. Jour. Bot. 31: 11–19. 1944.
5. BONNER, J., and S. G. WILDMAN. Enzymatic mechanisms in the respiration of spinach leaves. Arch. Biochem. 10: 497–518. 1946.
6. CALLAN, T., and J. A. R. HENDERSON. A new reagent for the colorimetric determination of minute amounts of copper. Analyst 54: 650–653. 1929.
7. COOK, H. T. Spinach and cabbage seed treatment. Virginia Truck Exp. Sta. Bul. 96: 1491–1510. 1937.
8. ——————. Vegetable seed treatment experiments and practices in Virginia. Proc. Assoc. Off. Seed Anal. N. Amer. 1937: 105–111. 1938.
9. ——————, and J. A. CALLENBACH. Spinach seed treatment. Virginia Truck Exp. Sta. Bul. 87: 1213–1233. 1935.
10. CROSIER, W. F., and S. PATRICK. Chemical elimination of saprophytes during laboratory germination of seed peas. Jour. Agr. Res. [U.S.] 58: 397–422. 1939.
11. ——————, and ——————. The value of chemical seed treatments in germination studies. Proc. Assoc. Off. Seed Anal. N. Amer. 1937: 117–121. 1938.
12. CROXALL, H. E., and L. OGILVIE. Experiments with protectant seed dressings. Bristol Agr. Hort. Res. Sta. Ann. Rept. 1942: 65–76. 1943.
13. ——————, and ——————. The incorporation of growth hormones in seed dressings. Jour. Pomol. Hort. Sci. 17: 362–384. 1940.
14. DAVIS, B. H., and C. M. HAENSELER. Tests with some vegetable seed protectants. U. S. Dept. Agr., Pl. Dis. Reptr. 27: 170–173. 1943.

15. DAVIS, G. N. Germination of treated and untreated pea seeds in autoclaved and unautoclaved soil. Proc. Assoc. Off. Seed Anal. N. Amer. 1937: 121–125. 1938.
16. ERWIN, L. E., and F. K. CRANDALL. Seed treatment studies of spinach. (Abstr.) Phytopath. 24: 8. 1934.
17. FIRKET, M. I., and MLLE. COMHAIRE. Experimental investigations on the glutathione contents of peas at the beginning of germination. Bull. acad. roy. méd. Belg. V, 9: 93–122. 1929. (Abstr. in Chem. Abst. 23: 3951. 1929.)
18. FLEMING, R. Modification de la reaction au nitroprussiate de soude pour les derives sulfhydryles a faible concentration. Compt. rend. soc. biol. (Paris) 104: 831–832. 1930.
19. GOULD, C. J. Vegetable-seed treatment tests in western Washington, 1943. U. S. Dept. Agr., Pl. Dis. Reptr. 27: 594–601. 1943.
20. HARRISON, D. C. The catalytic action of traces of iron and copper on the anaerobic oxidation of sulfhydryl compounds. Biochem. Jour. 21: 335–346. 1927.
21. HOPKINS, F. G., and E. J. MORGAN. Appearance of glutathione during the early stages of the germination of seeds. Nature 152: 288–290. 1943.
22. —————, —————, and C. LUTWAK-MANN. The influence of thiol groups in the activity of dehydrogenases. II. Biochem. Jour. 32: 1829–1848. 1938.
23. HORSFALL, J. G. Red oxide of copper as a dust fungicide for combating damping-off by seed treatment. N. Y. (Geneva) Agr. Exp. Sta. Bul. 615. 1932.
24. —————, A. G. NEWHALL, and C. E. F. GUTERMAN. Dusting miscellaneous seeds with red copper oxide to combat damping-off. N. Y. (Geneva) Agr. Exp. Sta. Bul. 643. 1934.
25. KADOW, K. J., and H. W. ANDERSON. Damping-off control: An evaluation of seed and soil treatments. Ill. Agr. Exp. Sta. Bul. 439: 291–348. 1937.
26. KANDA, M. Studien über die Reizwirkung einiger Metallsalze auf das Wachsthum höherer Pflanzen. Article 13 in Jour. Col. Sci. Imp. Univ. Tokyo 19: 1–47. 1904. (Abstr. in Exp. Sta. Rec. 16: 228. 1904.)
27. KEILIN, D., and T. MANN. Polyphenol oxidase. Purification, nature and properties. Proc. Roy. Soc. London, B, 125: 187–204. 1938.
28. KOTOWSKI, F. Chemical stimulants and germination of seeds. Amer. Soc. Hort. Sci. Proc. 23: 173–176. 1926.
29. LYMAN, D. M., and E. S. GUZMAN-BARRON. Studies on biological oxidations. VIII. The oxidation of glutathione with copper and hemochromogens as catalysts. Jour. Biol. Chem. 121: 275–284. 1937.
30. MACHLIS, L. The influence of some respiratory inhibitors and intermediates on respiration and salt accumulation of excised barley roots. Amer. Jour. Bot. 31: 183–192. 1944.
31. MERRY, J., and D. R. GODDARD. A respiratory study of barley grain and seedlings. Proc. Rochester Acad. Sci. 8: 28–44. 1941.
32. MUNN, M. T. Toxic effect of certain seed treatments as revealed in germination response. Proc. Assoc. Off. Seed Anal. N. Amer. 1936: 92. 1936.
33. PERSON, L. H. Seed and soil treatments for combating damping-off of tomatoes, eggplants and peppers. Proc. Assoc. Southern Agr. Workers 40: 180. 1939.
34. —————, and S. J. P. CHILTON. Seed and soil treatment for the control of damping-off. La. Agr. Exp. Sta. Bul. 349. 1942.
35. PIRIE, N. W. The oxidation of sulfhydryl compounds by hydrogen peroxide. I. Catalysis of oxidation of cysteine and glutathione by iron and copper. Biochem. Jour. 25: 1565–1579. 1931.
36. PIRONE, P. P., A. G. NEWHALL, W. W. STUART, J. G. HORSFALL, and A. L. HARRISON. Copper seed treatments for the control of damping-off of spinach. N. Y. (Cornell) Agr. Exp. Sta. Bul. 566. 1933.
37. PORTER, R. H. Detection and classification of seed-borne organisms, their effect on germination and their control by seed disinfection in laboratory and field. Proc. Assoc. Off. Seed Anal. N. Amer. 1938: 195–213. 1938.
38. ROBINSON, E. S., and J. M. NELSON. The tyrosine-tyrosinase reaction and aerobic plant respiration. Arch. Biochem. 4: 111–117. 1944.
39. SCHOBERL, A. Zur Kenntnis der Oxydation von Sulfhydrylverbindungen mit Wasserstoff superoxyd 1. Die Oxydation von Cystein mit Wasserstoff superoxyd. In Hoppe-Seyler's Zeitschr. Physiol. Chem. 209: 231–238. 1932.
40. SUMNER, J. B., and G. F. SOMERS. Chemistry and methods of enzymes. 365 pp. Academic Press, Inc., New York. 1943.
41. VANDEVELDE, A. J. J. Uber den Einfluss der chemischen Reagentien und des Lichtes auf die Keimung der Samen. Bot. Centbl. 69: 337–342. 1897.
42. WEIDENHAGEN, R., and F. HEINRICH. Tyrosinase of Beta vulgaris. Zeitschr. Ver. deut. Zuckerind. 1928: 499–538. 1928. (Abstr. in Chem. Abst. 23: 1664. 1929.)

ISOPROPANOL–SOLUBLE COMPOUNDS IN CONTROLLING STEM–END DECAY OF ORANGES

E. A. SIEGLER AND J. F. L. CHILDS[1]

Control of citrus decays with several water-soluble substances has recently been reported.[2] Experience with thiourea, however, focused attention on the practical importance of the toxicological properties of the compounds to be employed in stem-end-rot control because it was found that measurable amounts of this compound penetrated into the juice[2] under the conditions necessary for decay control.

In investigations to date, attention has been confined largely to water-soluble materials, presumably because of the expense of other solvents. This consideration should not necessarily preclude the trial of water-insoluble compounds. The penetration of water-insoluble compounds into the juice might be considerably less than that of water-soluble materials. It is obvious that the use of such materials, provided a suitable solvent could be found, would have the additional advantage of affording a wider range of compounds for testing. It therefore seemed advisable to change the approach to the problem by directing attention to the possibilities of water-insoluble compounds.

The experiments with water-insoluble compounds reported here cover a period from April to July, 1946, and are very limited in scope. The solvents used in preliminary tests were amyl acetate, benzene, butanol, isobutanol, butylamine, isopropanol, isopropanolamine, and combinations of these with water. Isopropanol (isopropyl alcohol) was selected for use as the solvent in the tests reported here because of its low cost and relative safety from the standpoint of rind injury.

Among the compounds tested, diphenyl sulfoxide and benzhydrol from General Chemical Company, and phenylurethane from American Chemical Paint Company, were found to be fungicidally or fungistatically active against the stem-end-rot fungi, *Diplodia natalensis* and *Phomopsis citri*. The compounds that did not prove effective in one or more of these tests were diphenyl, pinacol, diphenylmethoxy 2-methoxyethylether, tri-n-hexyl-citrate, furfurylidienemethyl isobutylketone, di-2-ethylhexyl carbonate, diphenylamine, sodium dimethyldithiocarbamate, calcium ethylene-bisdithio-carbamate, "lorol" pyridinium chloride (a mixture of 8–18 carbon atom fractions with the 12 or lauryl fraction occurring in highest percentage), "myristyl blend" pyridinium chloride (largely the C_{14} fraction), and bis-(octadecyl amino) cupric chloride.

The oranges available were limited to the Valencia variety by the season at which the experiments were conducted in Florida. Clipping was the principal method of harvesting, but occasional lots of fruit purchased at the packing houses had been pulled. Oranges used in a single experiment were either all clipped or all pulled, in recognition of the difference in the incidence of stem-end rot and Penicillium decay between the two types of picking. The tests conducted with this fruit were concerned primarily with control of stem-end decay. Due to light infection, a good test of control of green mold (*Penicillium digitatum*) or blue mold (*P. italicum*) was not obtained. After washing, the oranges were gassed with ethylene for approximately 40 hours before being treated. The method of treating the fruit differed slightly from previous tests in that the fruits were immersed

TABLE 1.—*The control of stem-end rot with diphenyl sulfoxide and benzhydrol in 100 per cent isopropanol, 30 seconds' immersion*

Treatment	No. exp'ts	Total number fruit	Percentage decay			Total rot (per cent)
			S.E.R.[a]	Pen.[b]	Other	
5 per cent Diphenyl sulfoxide	7	328	4.3	6.1	1.8	12.2
5 per cent Benzhydrol	7	320	2.5	1.6	1.3	5.3
Check	13	604	45.9	8.1	0.2	54.1
2.5 per cent Diphenyl sulfoxide	8	357	20.4	1.7	2.2	24.4
2.5 per cent Benzhydrol	7	327	14.1	3.7	0.3	18.0
Check	13	596	41.8	8.7	1.0	51.5
1.25 per cent Diphenyl sulfoxide	5	223	23.3	3.1	2.2	28.7
1.25 per cent Benzhydrol	4	181	19.3	2.2	1.7	23.2
Check	9	407	44.0	11.8	1.0	56.8
100 per cent Isopropanol	4	180	63.3	10.0	2.8	76.9
Check	4	181	47.5	17.7	0.6	65.9

[a] S.E.R. signifies stem-end rot caused by *Diplodia natalensis* or *Phomopsis citri*.
[b] Pen. signifies decay caused by *Penicillium digitatum* or *P. italicum*.

in the various solutions for 30 seconds (except where noted) instead of the usual 2 to 5 seconds. They were then allowed to dry and were stored at 70° F. for three weeks, when final inspection was made.

Diphenyl sulfoxide and benzhydrol were compared at 5 per cent concentration in 100 per cent isopropanol in seven experiments totaling 1252 fruits. As shown in table 1, diphenyl sulfoxide gave good control of stem-end rot (4.3 per cent decayed) and benzhydrol gave slightly better control (2.5 per cent decayed), compared with 45.9 per cent stem-end decay in the check lots. Lower concentrations of diphenyl sulfoxide or benzhydrol in isopropanol resulted in a material decrease in control (Table 1).

Although these two compounds are only slightly soluble in water, more than 2.5 per cent of either will stay in solution in 50 per cent isopropanol. The results of four experiments with these materials at this concentration show (Table 2) 8.2 per cent stem-end rot in the fruit treated with diphenyl

TABLE 2.—*The control of stem-end rot with diphenyl sulfoxide and benzhydrol at 2.5 per cent concentration in 50 per cent isopropanol, 30 seconds' immersion*

Treatment	No. exp'ts	Total number fruit	Percentage decay			Total rot (per cent)
			S.E.R.[a]	Pen.[b]	Other	
Diphenyl sulfoxide	4	194	8.2	5.7	2.1	16.0
Benzhydrol	4	182	3.8	2.2	1.1	7.1
Check	7	324	41.4	8.6	1.2	51.2

[a] S.E.R. signifies stem-end rot caused by *Diplodia natalensis* or *Phomopsis citri*.
[b] Pen. signifies decay caused by *Penicillium digitatum* or *P. italicum*.

sulfoxide, 3.8 per cent in the lots treated with benzhydrol, and 41.4 per cent in the checks. Thus it appears that although a concentration of 2.5 per cent of these compounds in 50 per cent isopropanol is less effective than 5.0 per cent in 100 per cent isopropanol, it offers promise from the standpoint of practical utilization.

Phenylurethane gave results somewhat superior to the other two compounds. Ten experiments with phenylurethane at several concentrations in isopropanol (100 per cent), in which the fruits were immersed 2 to 5 seconds in the solutions, are summarized in table 3. Excellent control was obtained at 5 per cent concentration; the treated lots showed 1.0 per cent stem-end rot in contrast to 34.0 per cent in the check lots. At lower concentrations phenylurethane was not effective.

Isopropanol (50 per cent) was used as a solvent for phenylurethane (5 per cent concentration) in a series of four experiments totaling 452 fruits. The average total decay was 1.1 per cent in the treated lots and 40.2 per cent in the check lots. In very limited tests with 2.5 per cent phenylurethane in 50 per cent isopropanol the degree of control was materially reduced.

To determine the effect of the solvent on the incidence of decay, four lots (total 180 fruits) were immersed 30 seconds in isopropanol and four paired samples (total 181 fruits) were not treated. Variability of the

TABLE 3.—*The control of stem-end rot with phenylurethane in 100 per cent isopropanol, 2 to 5 seconds' immersion*

Treatment	No. exp'ts	Total number fruit	Percentage decay			Total rot (per cent)
			S.E.R.[a]	Pen.[b]	Other	
5 per cent Phenylurethane ..	7	306	1.0	0.0	1.9	2.9
Check	9	406	34.0	6.9	1.5	42.4
2.5 per cent Phenylurethane	6	286	7.7	6.6	0.7	15.0
Check	6	281	17.8	5.0	1.4	24.2
1.25 per cent Phenylurethane	5	219	13.2	14.6	1.4	29.2
Check	6	257	17.1	3.5	1.9	22.6

[a] S.E.R. signifies stem-end rot caused by *Diplodia natalensis* or *Phomopsis citri*.
[b] Pen. signifies decay caused by *Penicillium digitatum* or *P. italicum*.

results (Table 1) was such that the differences in amount of stem-end rot or of total decay due to treatment were not statistically significant.

The possibilities of decay control with these compounds when incorporated in emulsified oil or in polishing waxes have not been investigated. However, in a single experiment, these three materials were used at 5 per cent concentration in a light summer oil and applied to oranges by the dip method. At three weeks the fruit treated with diphenyl sulfoxide showed 10.2 per cent total decay with moderate rind burn, and that treated with benzhydrol showed 11.1 per cent total decay with rather severe rind burn. Phenylurethane treatment gave 2.4 per cent total decay with only slight burns where the oil had gathered at spots of contact between fruits. There was 22.0 per cent total decay in the check lots.

The field of nonaqueous solvents for fungicides is one that has received little attention, but, judging from the above results, it merits further investigation. Some compounds that have been ineffective when used with water may prove effective in nonaqueous solvents; other effective compounds, used either in solution or dispersed in nonaqueous solvents, may prove more acceptable because of possible restricted tissue penetration.

U. S. SUBTROPICAL FRUIT FIELD STATION,
	ORLANDO, FLORIDA

WATER–CONGESTION AND FUNGUS PARASITISM

JAMES JOHNSON

Field observations and laboratory experiments have shown that the accumulation of excessive water in the intercellular spaces of plant tissues is relatively common under conditions favorable for its occurrence. This form of natural water-congestion arises chiefly from sources internal to the plant and hence does not include water entering tissues through wounds. The latter condition involves an additional set of factors which would complicate the problems under present consideration. The direct relation between water-congestion, host predisposition, and varietal susceptibility to certain bacterial parasites has been previously reported (2, 6). Experiments on water-congestion and fungus parasitism appeared at first more uncertain and complicated, because the conditions required for spore germination and the methods of host-penetration were not so simple as with bacterial parasites. It was shown, however, that detached, artificially water-congested unwounded leaves are readily invaded by fungi as compared to uncongested leaves under the same conditions (3).

Further progress in this study seemed to depend on inducing natural water-congestion at will in an experimental moist chamber. This is not always easily accomplished even though congestion often occurs without an intentional effort. Furthermore, since the conditions required for spore germination and infection are similar to those required for water-congestion it became necessary, for comparative purposes, to seek conditions and plants which failed to develop congestion under the same environment that favored congestion of other material. This necessitated some preliminary experiments and modifications of the moist chamber so that the soil and air environment could be varied and controlled. Detecting soils that differed in ability to yield water-congestion, and varieties responding differently in this respect was especially important. Later it was found that greenhouse-grown plants placed outdoors for a few days prior to inoculation commonly developed higher susceptibility to water-congestion and infection than did plants grown continuously in the greenhouse (4). Such variations in treatment were often applied simultaneously in order to secure comparative data on both water-congestion and infection.

The results of these studies, although not presented in detail, are believed sufficient to establish a connection between water-congestion and certain fungus diseases. Consequently they may explain a part of the frequently observed erratic behavior of some fungus diseases under natural conditions where the parasite is known to be present in abundance. An abstract of this paper has been previously published (5).

METHOD OF INVESTIGATION

Among the basic factors involved in water-congestion are the potash content of the soil, the genetic variation in varieties of plants, and the environment under which the plants grow just prior to exposure to favorable conditions for congestion. These and other conditions may be varied in a number of different ways. For example, the age of the plants at the time of the treatments, the duration of the treatments, and the soil and air temperature at which these treatments are applied permit almost unlimited possibilities of modification in the trials. Efforts were usually made to hold as many factors constant as possible while varying only one or two factors at a time. This is often difficult with limited amounts of equipment, such as only one moist chamber, but it has nevertheless been possible to often obtain two groups of plants otherwise comparable, one of which water-congested heavily and the other of which was not visibly congested, at the same time retaining conditions suitable for infection.

The best water-congestion has been secured with sandy-loam farm soils low in potash. Fertile greenhouse compost usually fails to give visible congestion. The number of days that plants were grown outdoors in comparison to greenhouse-grown plants was often varied, but some limitations arose here, as, for example, the periods of the year when such treatment might be applied in a northern climate with highly variable temperatures. In our trials, outdoor exposures were limited to relatively short periods during the spring and fall seasons. The summer period was not adapted for such studies because of the high temperatures prevailing in the greenhouse moist chamber. The plants were exposed outdoors in an open cold frame, which could be covered at night and during rains to prevent possible wounding by storms or probable excess precipitation into the soil containers.

The moist chamber was provided with thermostatic air control and thermostatic bottom heat to provide wider differential soil and air temperatures if desired. Galvanized iron soil containers (Fig. 1) permitted more rapid increases in soil temperature when bottom heat was applied. The soil or air temperatures could be varied between about $14°$ and $34°$ C. The moisture content of the atmosphere of the chamber was the most constant factor, saturation being maintained by fine oil-nozzle atomizers directed away from the plants and so regulated as to produce a fog only sufficient to maintain a constant film of moisture on the plant surfaces.

The time of exposure in the moist chamber rarely exceeded 24 hours, frequently being 18 or as little as 4 to 6 hours, the latter often yielding infection with some pathogens. Exposures as short as one hour yielded good water-congestion with some species, although, for reasons not fully determined, 24 hours frequently failed at other times to yield visible congestion on the same species. The highly complex nature of water-congestion is obvious, but the physiological sensitivity of the plant to water-congestion is no more erratic in our experience than is the response of many plants to infection under either artificial or natural field conditions.
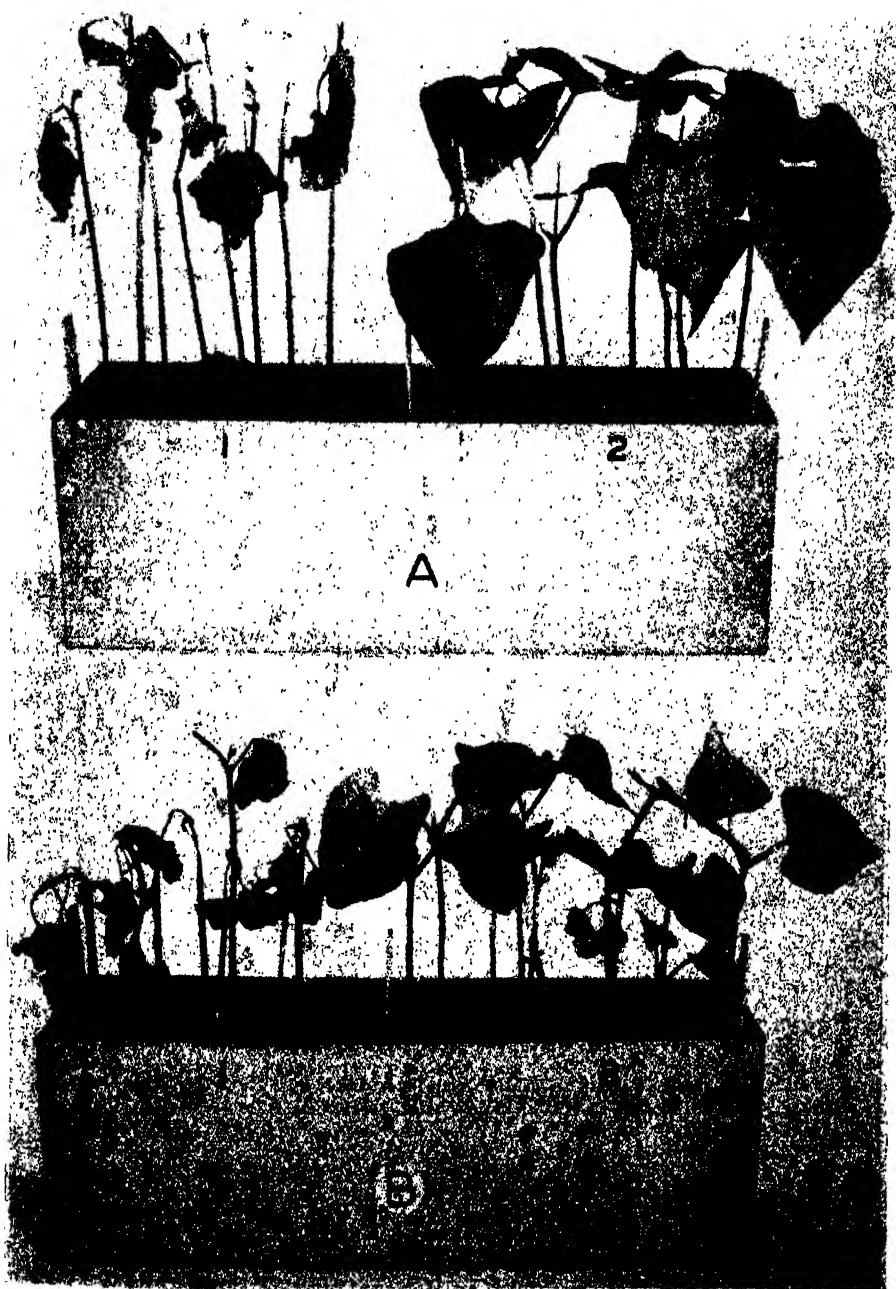
FIG. 1.   Bean anthracnose on a susceptible bean variety, Bountiful (1), and on a resistant variety, Great Northern (2), grown continuously in the greenhouse (A) or placed outdoors for 3 days prior to inoculation (B).   Note numerous leaf lesions in B, 2 as contrasted with the scarcity of lesions in A, 2.   Metal soil containers used facilitated bottom heating.   Photograph by Eugene H. Herrling.

EXPERIMENTAL RESULTS

For this investigation, it seemed desirable to use certain common and representative types of fungi parasitic on readily grown hosts. The results on 7 such diseases are reported here.

Bean Anthracnose caused by Colletotrichum lindemuthianum (Sacc. and Magn.) Bri. and Cav.—The garden bean (*Phaseolus vulgaris* L.) is an ideal plant for experiments on water-congestion because of its rapid growth, the number of varieties available, and the ease with which it may be water-congested. The varieties used have included the white (Navy, Great Northern, and White Marrow), the brown or pink (Giant Stringless Green Pod, Bountiful, Wisconsin Refugee), and the black (Black Wax, Black Wax Valentine).

The experiments with beans were confined largely to the primary leaf stage. These leaves congest rapidly and heavily under the proper conditions. Visible congestion often was noted after one hour, and in 6 to 12 hours almost the entire leaf area of some plants became fully congested. It has been observed in all trials that all plants of a single variety do not congest with equal ease. Some individuals appear to be relatively resistant and others relatively susceptible to water-congestion. Bean varieties normally differ greatly in susceptibility to congestion. The resistant varieties may become heavily congested, however, under prolonged favorable conditions.

It has been noted repeatedly that bean plants grown outdoors, especially at relatively low temperatures, congest more easily than plants grown indoors. This response is partly a temperature relation as may be shown by comparing indoor plants grown at low (65–70° F.) and high (75–80° F.) greenhouse temperatures. However, as far as the amount of subsequent infection on water-congested leaves is concerned pretreatment at low greenhouse temperatures is not so effective as exposure outdoors. The beans were sown and started in the greenhouse, usually at higher average temperatures than those encountered for a 5 to 10 day period outdoors. With this method it was not always possible to secure greenhouse-grown and outdoor-grown plants of equal size or stage of growth, but often the differences were small and perhaps unimportant in the results secured.

Inoculations with *Colletotrichum* were first made with conidia from culture but in later inoculations conidia were taken from diseased stems and petioles placed in a small moist chamber for 24–48 hours for sporulation. The physiologic strain of the parasite used was not determined. The inoculum was in all cases a very heavy spore suspension applied with a DeVilbiss atomizer held a foot or more from the plants so as not to wound the epidermis or force the spores through stomatal openings. The time of inoculation varied, but usually the inoculum was applied just before the plants were set in the moist chamber. Much infection was sometimes secured on anthracnose-susceptible varieties after 12-hour exposures, and

at other times only poor infection was secured after 24-hour exposures. When water-congestion was rapid and extensive, infection was invariably heavy on leaf blades, veins, petioles, and upper and lower portions of the hypocotyl. Heavy petiole and hypocotyl infection usually resulted in early collapse of the leaves even though the number of infections of the leaf blades was relatively light. Occasionally round leaf-spot or linear vein lesions developed on the leaves only, and these sometimes occurred in the absence of good visible signs of water-congestion.

To compare amounts of infection, it was desirable to secure both good water-congestion and no congestion following the same exposure in the moist chamber. This frequently could be accomplished by utilizing selected varieties or selected soils responding differently to water-congestion, or by using outdoor and greenhouse grown plants of the same variety in the same soil. In addition it is desirable to limit the time of exposure in the moist chamber to the minimum required for infection. Many other conditions were tested, such as variations in soil moisture, soil and air temperature, light, age of plant, pre-exposure in the moist chamber prior to inoculation, etc., but the results have been less consistent than those obtained by the other methods described. The more basic factors involved in heavy infection appeared to depend upon the presence of water-congestion together with a conditioning of the epidermis or cuticle favoring penetration. These factors in turn were influenced largely by the environment in which the plants were grown a few days prior to inoculation and exposure in the moist chamber. Consequently it does not follow that all water-congested tissue is uniformly susceptible to penetration by the parasite, or that tissues which are not macroscopically congested may not be penetrated. It has been shown previously that water congestion may be so slight that it can be detected only with the aid of a microscope; yet it may be a predisposing factor to leaf penetration by pathogens (3).

Varietal differences to water-congestion are often striking, even though some varieties of beans are evidently heterozygous for this character. It is difficult to compare our results, with an undetermined physiologic strain of the parasite, with variety tests made by others in the field or greenhouse under less specific conditions as to environment or method of inoculation. No varieties of beans are said to be immune from anthracnose if tested under severe conditions, but marked differences in disease resistance have been reported under both field and greenhouse conditions. Such varietal differences have been sought for use in our tests. White Navy and Great Northern repeatedly have been noted to water-congest less readily and to be more resistant to anthracnose than Giant Stringless Green Pod, Black Wax, Bountiful, and Refugee, and yet they may become heavily congested and severely infected under favorable conditions (Fig. 1). White Marrow, although penetrated and heavily flecked with anthracnose, never has been extensively water-congested or diseased in our trials. Points of penetration of the parasite on this variety often appear to be even more numerous than

on varieties very susceptible to the disease. The congestion and penetration on Marrow appears to be limited largely to the leaf veins, where incipient superficial lesions develop in especially large numbers and eventually coalesce into larger discolored streak areas, but intensive necrosis is rare.

The conclusions relative to bean anthracnose are based on 56 series of inoculations with *Colletotrichum lindemuthianum*, and, although the results have varied greatly with the method of treatment, all show that host-predis-

TABLE 1.—*The relation of water congestion to development of anthracnose on bean plants grown continuously in the greenhouse or given an outdoor pretreatment prior to inoculation without wounding*

Exp.	Incubation		Pretreatment		Host variety	Early signs of infection[a] on					Final development of disease[a]
	Hours	Temp., °C.	Days	Place		Visible water-congestion[a]	Leaf-blades	Veins	Petioles	Stems	
1	24	20–23	7	Outdoors	Bountiful	+++	+	+	+	+	+++
					White Marrow	+	0	+	0	0	+
				Indoors	Bountiful	0	0	0	0	0	++
					White Marrow	0	0	0	0	0	+
2	24	18–20	7	Outdoors	Bountiful	++	+	+	+	+	++++
					White Marrow	+	0	+	0	0	+
				Indoors	Bountiful	+	0	+	+	0	++
					White Marrow	+	0	0	0	0	+
3	24	21–25	7	Outdoors	Black Wax	++	+	+	+	+	++++
					White Marrow	+	0	+	0	0	+
				Indoors	Black Wax	0	+	+	+	0	++
					White Marrow	0	0	+	0	0	+
4	15	22–24	3	Outdoors	Bountiful	++	+	+	+	+	++++
					Great Northern	++++	+	+	0	0	+++
				Indoors	Bountiful	++	+	+	+	+	++
					Great Northern	+	+	0	0	0	+
5	20	19–25	8	Outdoors	Black Valentine	++	+	+	+	+	++++
					Great Northern	+	+	+	+	0	++
				Indoors	Black Valentine	++	+	+	+	+	++
					Great Northern	+	0	0	0	0	+

[a] Amount of water-congestion, infection, or disease: 0 = none visible; + = small; ++ = moderate; +++ = heavy; ++++ = very heavy.

position and relative susceptibility of bean varieties to anthracnose may be altered by conditions which bring about variation in water-congestion (Table 1). This conclusion does not contradict previous observations of others that such conditions as stage of maturity and other gross factors are involved in anthracnose infection; it rather offers a possible explanation for such behavior. Not only the stage of maturity but the particular plant organ attacked may influence the results and interpretation of experiments designed to vary host-predisposition. Although varietal variation to anthrac-

nose infection may be explained on the basis of water-congestion, it seems likely that still other factors are involved in the later stages of disease development.

Potato Late-Blight (*Phytophthora infestans* (Mont.) *de B.*).—Visible signs of water-congestion rarely were obtained on the leaves of potato in the moist chamber, although it can be demonstrated to be present by submerging the leaves in rose bengal dye. The potato leaf may be congested readily by artificial water pressure and, in common with many other species of plants, is able to take up 20 per cent[1] or more of its original weight as congestive water. In the potato it appears that microscopic or invisible amounts of water-congestion in tissues are sufficient to favor infection by *Phytophthora;* but, without any water-congestion, infection evidently does not occur.

Following the usual method of inoculation, little difficulty was experienced in securing severe expressions of the disease which eventually resulted in the death of the plants. Varying the ordinary environmental factors under which the plants were grown prior to inoculation appeared to have little effect on subsequent disease development. Varieties reported to have some resistance (Sebago, Sequoia) collapsed as rapidly as Triumph, Red Warba, Chippewa, and Katahdin varieties (Table 2). It then became evident that an exposure of 24 hours in the moist chamber was so favorable to infection that all varieties become about equally infected. Furthermore, it also was obvious that conclusions on penetration should be based only on the number of original points of infection and not on later stages of blight development. Potato late-blight, even in the greenhouse, continues to spread into new tissues, with the result that the entire plant may succumb from relatively few points of infection.

With the potato, therefore, it seemed desirable to lower the time of exposure sufficiently to prevent such heavy infections as tend to mask both variations in host-predisposition and relative varietal resistance to disease. When the time of exposure in the moist chamber was lowered to 6, 9, or 12 hours, other conditions being favorable, the number of necrotic lesions produced at the points of infection were fairly proportional to the relative resistance and susceptibility of the varieties used. Following short exposures in the moist chamber, plants grown outdoors 5 to 10 days prior to inoculation were more predisposed to infection than greenhouse-grown plants, even though visible signs of water-congestion were not evident. This response nevertheless supports the conclusion that congestion was present, though not visible. The significance of water-congestion to infection of potato with *Phytophthora* is regarded, however, as being more dependent on interpretation than is the case of bean anthracnose.

Tomato Late-Blight (*Phytophthora infestans* (Mont.) *de B.*).—The behavior of *Phytophthora* on tomatoes is more easily interpreted on the water-congestion basis. No difficulty was experienced in securing heavily infec-

[1] The data dealing with the amount of water-congestion, based on percentage increase in weight of plant tissue, will be presented in a separate paper.

tions with the potato strain of the fungus. The tomato congests easily; the number of infection points may be far more numerous than on the potato with the same inoculum, but the subsequent development of the disease in the greenhouse is more limited to the original infected areas and the host hence tends to recover from heavy infections more readily than is the case with potato.

TABLE 2.—*The relation of water-congestion to infection and disease development in 4 rusts and 2 late blight diseases on plants grown indoors or given a pretreatment outdoors prior to inoculation without wounding*

Disease	Incubation		Pretreatment		Host variety	Visible water-congestion[a]	Early signs of infection[a]	Final development of disease[a]
	Hours	Temp., °C.	Days	Location				
Wheat stem rust	24	19–21	6	Outdoors	Marquis	++++	++	+++
					Henry	++++	+	+
				Indoors	Marquis	+	+	++
					Henry	++	0	⊤
Oat leaf-rust	12	19–21	8	Outdoors	States Pride	++	+	+++
					Vicland	+	++	+++
				Indoors	States Pride	0	+	++
					Vicland	0	+	+++
Corn rust	6	18–20	5	Outdoors	Golden Bantam (sweet)	+++	++	++++
					Golden Glow (field)	+	+	+++
				Indoors	Golden Bantam	0	+	+
					Golden Glow	0	+	+
Potato late-blight	6	18–23	7	Outdoors	Triumph	0	+	++
					Sebago	0	++	+++
				Indoors	Triumph	0	0	+
					Sebago	0	0	+
Tomato late-blight	7	20–22	10	Outdoors	Marglobe	++	++	++++
				Indoors	Marglobe	0	+	+
Sunflower rust	12	18–22	14	Outdoors	Mammoth Russian	0	++	++++
				Indoors	Mammoth Russian	0	+	++

[a] Amount of water-congestion, infection, or disease: 0 = none visible; + = small; ++ = moderate; +++ = heavy; ++++ = very heavy.

As a result of this behavior, it is frequently possible to observe first the marginal and angular water-congested leaf-areas, and to note subsequently their relation to the points of infection, and to the limits of progress of the disease in these areas. When the thin tomato leaves return to normal water relations, the progress of the invasion appears to be sharply halted, as contrasted to the reaction on the potato. The results with *Phytophthora* on

tomato (Table 2) are in line with those secured with bean anthracnose, and are more readily demonstrated and interpreted than the results with potato.

Barley Leaf-Blotch (Helminthosporium sativum P. K. and B.).—Among 35 species of plants tested, including mostly common crop plants, barley was the most susceptible to natural water-congestion. It was more difficult to congest greenhouse grown barley plants at will during the winter months, and typical or heavy infections on the leaves were not obtained from inoculations with *Helminthosporium*, although good varietal differences in stem infections (foot-rot) were obvious. It was not certain, however, that the spores from the cultures possessed an equal degree of infectivity. These and other factors may account for the relatively inconsistent results secured with leaf-blotch of barley. In two out of several trials, however, obvious correlations between water-congestion and infection occurred in outdoor and greenhouse grown plants, and between resistant (Oderbrucker Wis. Pedigree 5) and susceptible (Wis. Barbless Pedigree 38) barleys.

Leaf-Rust of Oats (Puccinia coronata Corda).—Oats are less susceptible to water-congestion than some other grains, but little difficulty was experienced in securing good congestion under favorable conditions. Proper conditions again involved chiefly the selection of a suitable soil and pre-exposure of plants outdoors for 5–10 days prior to exposure in the moist chamber. As a rule, the tests were conducted in the early seedling stage. The varieties most frequently used were States Pride (rust-susceptible) and Vicland (rust-resistant). The strain of leaf rust originally used was collected from a local field of the States Pride variety. An abundance of young spores from previously infected plants was usually available for inoculations. Inoculations were made with water suspensions from an atomizer, since this method appeared to offer better possibilities than spore dusting for a uniform distribution of spores over the leaf surfaces. The usual period allowed in the moist chamber was 12, 18, or 24 hours.

Flecks and pustules were always more numerous on plants that had been exposed outdoors than on plants continuously indoors. Frequently excellent infection was secured on outdoor plants and none on indoor plants (Fig. 2). The number of flecks or pustules was not necessarily proportional to the amount of water-congestion, however; and, as in the case of bean anthracnose, it is clear that other factors in the epidermal layer or the cuticle, especially the stomatal position, are highly important to infection when associated with water-congestion. It seems fairly certain from several series of trials that little or no infection from leaf-rust of oats developed in the absence of water-congestion.

Of special interest in the trials with oats was the relative infection secured on normally resistant and susceptible varieties when the pretreatments were varied before inoculation and confinement in the moist chamber. Commonly, States Pride (susceptible) congested earlier and yielded heavier infection than Vicland (resistant). In some trials, however, Vicland appeared to be more susceptible than States Pride as far as penetration

and early infection were concerned (Table 2). Nevertheless, Vicland rarely yielded more or larger pustules than States Pride. Vicland was obviously more resistant to subsequent progress of the fungus, indicating that more than one factor is concerned with disease resistance to leaf-rust in oats. It

FIG. 2. Leaf rust on a susceptible variety of oats, States Pride, grown continuously in the greenhouse (A) or grown outdoors for 7 days prior to inoculation (B), and on a resistant variety of oats, Vicland, grown outdoors for 7 days prior to inoculation (C). Photograph by Eugene H. Herrling.

is suggested that the intercellular spaces may be smaller in the resistant variety, thus they may retain less congestive-water and check the progress of the parasite earlier than in the susceptible variety.

Wheat Stem Rust (Puccinia graminis Pers.).—The strain of wheat rust used was not determined, but the original inoculum was secured from a nursery heavily infected with stem rust after artificial inoculation with race 56 of *Puccinia graminis tritici*, and the purity of the rust was presumably retained. However, the experimental results are based only on symptoms secured on either young seedlings or leaves of older plants prior to the heading stage. Four varieties of wheat were used; namely, Henry and a durum variety (resistant) and Progress and Marquis (susceptible). Henry and Marquis were most frequently compared.

As in the case of oats, water-congestion was regularly more pronounced on wheat plants grown outdoors some days prior to inoculation (Table 2). The amount of rust infection on spray-inoculated plants was, as a rule, fairly proportional to the amount of congestion. Occasionally, the amount of congestion on outdoor plants of the Henry variety (resistant) was more conspicuous than on Marquis, although the reverse was the case on indoor plants. In one trial this behavior was closely related to the amount of subsequent infection and pustule development, so that outdoor plants of the resistant variety appeared relatively susceptible and the indoor plants of the same variety appeared relatively resistant.

During the winter months when plants could not be grown outdoors prior to inoculation, the variation of other environmental conditions (including soil type, soil moisture, soil and air temperature, pre-exposure in the moist chamber prior to inoculation and other treatments) yielded no convincing differences in amount of infection. It was not until outdoor pretreatment could be combined with soil differences that significant variations in infection were obtained. It seems very likely that in our trials some of the erratic behavior toward infection in the presence of water-congestion is attributable to varietal differences in stomatal behavior as described by Hart (1).

In some of the trials with wheat, natural infection with wheat mildew (*Erysiphe graminis* D. C.) developed. This type of pathogen, which commonly develops under environmental conditions apparently not favorable to water-congestion, was nevertheless increased in severity by water-congestion of the host tissues.

Corn Rust (Puccinia sorghi Schw.).—Corn seedlings usually water-congest easily and quickly in a moist chamber, as they often do on greenhouse benches or in the field when the humidity is high. We have not noted as yet any striking or consistent differences in varieties in this respect, but only four have been grown in our tests. For the most part, Golden Glow (field corn) and Golden Bantam and Stowell's Evergreen (sweet corn) were used. Trials were made with some strains of sweet corn that are believed to vary in rust resistance, but no significant differences were found.

The corn rust originally was collected from the field and an abundance of inoculum was available thereafter from greenhouse plants. Good infections may be obtained with atomized spores after relatively short exposures (4-8 hours) in the moist chamber, although longer exposures often yielded heavier infection. A variety of pretreatments with greenhouse grown plants failed to yield significant variations in either the amount of water-congestion or the amount of infection.

It was not until the spring months, when normal corn plants could be grown outdoors, that host-predisposition to rust could be influenced to a significant degree. Corn plants grown outdoors (at a favorable temperature) for 5-10 days, and exposed in the moist chamber for a period as short as 6 hours at 18° C., congested very heavily and yielded 3 times as many pustules as greenhouse grown plants in one trial (Table 2).

By reducing exposure in the moist chamber to a time that was minimum for congestion and penetration in a susceptible variety but insufficient for congestion in a resistant variety, it was possible to obtain differences in rust infection on varieties of corn. Because field conditions seldom coincide with this experimental procedure, there may appear to be no differences in field reactions of these sweet corn strains to rust. Good infection has been obtained on corn in the presence of very little visible water-congestion, and it cannot be overemphasized that the amount of visible water-congestion alone is not necessarily correlated with the amount of fungus penetration.

Sunflower Rust (*Puccinia helianthi* Schw.).—Only one variety of sunflower (*Helianthus annuus* L.) was used. The fungus was obtained from a natural infection of plants in the field. Infection was so regular and extensive under the greenhouse conditions of the experiments that the period of exposure in the moist chamber was gradually reduced to 4 or 5 hours in order to secure significant variation in the development of the disease. However, visible water-congestion was not obtainable either with long exposure in the moist chamber or with widely varying pretreatments. The dye bath method of testing for the presence or absence of water-congestion also yielded irregular results. Sunflower leaves, however, may be easily and heavily congested with artificial water pressure. In general, it appeared that when moisture was maintained on the leaf surface for only 1 to 2 hours beyond the time required for spore germination, infection occurred without regard to any apparent predisposing condition of the host. Nevertheless, the variability of different leaves on the same plant and of different parts of the same leaf suggested that some undetermined factor was concerned with infection. It is probable that if varieties of sunflower differing in susceptibility had been available, further evidence on this point might have been obtained.

When sunflower plants grown outdoors for 5-10 days were compared to indoor grown plants, and the period of exposure in the moist chamber was sufficiently reduced, marked differences in amounts of infection were obtained (Table 2). The results sufficiently approached those secured with

other diseases to show that the outdoor influence on host-predisposition was not entirely absent. On the basis of these comparable trials, there is reason to believe that some excess water is present in the intercellular spaces when infection takes place.

DISCUSSION

Natural water-congestion plays a fundamental rôle in infection with certain bacterial diseases, especially with tobacco wildfire (2, 6). With artificial water-congestion of detached leaves of various species of plants, a probable relationship to infection with certain fungi was also indicated (3). Many field observations have supported the preliminary laboratory studies, but actual experimental evidence seemed to be required to secure further information. In this investigation, the chief difficulty has been to obtain differential degrees of water-congestion at will while maintaining other conditions identically favorable for infection by the pathogen. Unfortunately, water-congestion is induced by the same conditions that favor infection; consequently, some pretreatment must be relied upon to delay or prevent congestion under conditions which permit infection. Thus far, this has been frequently partially accomplished. Complete freedom from congestion is not readily determined.

Many other phases of the problem have arisen during the course of this study. It is clear that water-congestion alone, in the absence of wounds, is not always sufficient to permit penetration of a pathogen into host tissues. With the bacteria, the stomatal position and the condition and amount of the waxy cuticle are obviously associated factors. Many of the fungi with which we have worked are known to penetrate through the stomata, but with the bean anthracnose fungus it has been generally accepted that direct penetration of the epidermal cells by germ tubes from an appressorium is the normal method of infection. It is possible that both direct and stomatal penetration of the bean leaf may occur but that the relative frequency of each may depend upon environmental conditions. From results secured with the bean anthracnose fungus in the present investigation, it is believed that the method of penetration is partly stomatal because of the short periods of exposure required for infection in a favorable environment. Some of these periods apparently were shorter than those previously reported necessary for spore germination alone. Although our knowledge of this subject is still meager, it obviously is important that the optimal conditions for host penetration should be known before the predominating type of invasion can be satisfactorily determined. For present purposes we may need to assume only that the parasite enters the host through the path of least resistance. When the tissues are unwounded and water-congested, it seems likely that this path is through the stomatal openings, especially when the appressoria lie immediately above.

When all the factors of host-predisposition and variation in resistance

to disease are taken into account, as well as the influence of environmental conditions on the parasite itself, it is not difficult to understand the frequently observed erratic behavior of plant pathogens. The conclusion that water-congestion plays an important part in this behavior with some fungus diseases, as it does for certain bacterial diseases, seems to be justified.

SUMMARY

Natural water-congestion in many species of plants may be produced experimentally under favorable moist-chamber conditions. The plants water-congest most readily if grown outdoors in sandy or sandy-loam soils low in potash. For experimental purposes, it usually suffices to expose greenhouse grown seedlings to outdoor environment for 5–10 days prior to transfer to the moist chamber. With other soil types, or with plants not exposed outdoors, many species or varieties water-congest only very slowly. Thus, by limiting the time of exposure of inoculated plants in the moist chamber, it was possible to secure both heavily water-congested plants and plants not visibly congested during the same period of incubation.

Marked differences in rate and amount of visible water-congestion occurred between varieties and sometimes between individual plants of the same variety. Water-congestion developed in some species in as little time as one hour. In other species, e.g., the potato and the sunflower, visible natural water-congestion was not obtained in the moist chamber.

Plants of several species, following growth and pretreatment under varying conditions, including those described, were inoculated with representative fungus pathogens and incubated in the moist chamber under conditions favorable for water-congestion. Results are reported on several fungus diseases: bean anthracnose, potato late-blight, tomato late-blight, leaf-blotch of barley, leaf-rust of oats, wheat stem rust, corn rust, and sunflower rust.

It was found that water-congested plants of the same variety were more predisposed to infection by certain fungi than plants not water-congested. Varieties susceptible to disease congested more easily as a rule than varieties resistant to the fungus parasites in question. The amount of fungus penetration and infection was not always correlated with the amount of water-congestion. Other conditions not definitely determined, but favored by outdoor exposures as contrasted to continuous greenhouse culture, were involved.

The varied conditions of the experiments were such that the genetic resistance of some varieties often was obscured by heavy infection and the resistant and susceptible varietal characters apparently reversed.

Although potato and sunflower yielded no visible water-congestion in the moist chamber in these particular trials, good infection with late-blight and rust, respectively, was secured on both indoor and outdoor plants. More infection was secured on outdoor grown plants, and the results are believed to be associated with macroscopically invisible amounts of water-congestion.

Such a form of slight water-congéstion has been demonstrated microscopically, as well as by the use of dyes and by comparative weights.

UNIVERSITY OF WISCONSIN,
   MADISON, WISCONSIN.

LITERATURE CITED

1. HART, H.   Relation of stomatal behavior to stem rust resistance in wheat.   Jour. Agr. Res. [U.S.] 39: 929-948.   1929.
2. HEGGESTAD, H.   Varietal variation and inheritance studies on natural water-soaking in tobacco.   Phytopath. 35: 754-770.   1945.
3. JOHNSON, J.   Infection experiments with detached water-congested leaves.   Phyto path. 35: 1017-1028.   1945.
4. ——————.   Water-congestion and infection experiments on various plant species. (Abstr.) Phytopath. 36: 12.   1946.
5. ——————.   Fungus invasion of water-congested tissues.   (Abstr.) Phytopath. 37: 12.   1947.
6. ——————, W. B. ALLINGTON, and A. C. BRAUN.   Certain factors relating to the control of the blackfire and wildfire diseases of tobacco.   Wis. Agr. Exp. Sta. Res. Bull. 147.   1943.

PHYTOPATHOLOGICAL NOTES

A Rapid Method of Examining Wheat Heads for Bunt Infection.[1]—Bunt of wheat (*Tilletia foetida* (Wallr.) Liro, and *T. caries* (DC.) Tul.) is usually detected in the field by a visual inspection of the heads supplemented, whenever necessary, by an examination of individual spikelets for bunt balls. If the diagnosis is carried out by one who is familiar with the symptoms of bunt infection the presence or absence of bunted grains can be accurately determined. When the purpose of the examination is limited to the collection of bunted heads or where it is desired to determine only approximately the degree of bunt infection a visual examination of heads will usually suffice. It frequently happens, however, that only one or two spikelets in a head are bunted; so that, if the purpose of the examination is to determine accurately the degree of infection, it becomes necessary to inspect each individual spikelet. This requires considerable time and labor, especially in experiments involving the inoculation of a number of wheat varieties with several physiologic races of bunt. In such instances, the task of examining each individual spikelet in several thousands of heads places a definite limitation on the amount of experimental work that can be undertaken. To facilitate the detection of bunt some workers have tried clipping the individual florets of the heads whereas others have resorted to threshing each head individually and then examining the grain for the presence of bunt balls, but even these devices require considerable time.

The present note describes a method of detection based on the observation that bunted heads of ripening wheat are more conspicuous immediately following a drenching rain. The method is simple and has proved to be rapid and accurate.

The grain to be examined is harvested when the majority of the heads are ripe. The percentage bunt infection may be determined at once, or the grain may be stored and examined for infection at a later date. Immediately prior to examination the heads are soaked in water. Soaking may be done in either of two ways: (1) By immersing the heads in water at or near the boiling point and leaving them to soak overnight. (2) By immersing the heads for about 24 hours in water (at about room temperature) to which a wetting agent has been added.

The examination for bunt is carried out in a good light while the heads are still wet. The contrast in bunted heads before and after soaking in water is very striking (Fig. 1). When soaked the bunt balls become swollen and much darker. They can usually be recognized either through or between the glumes which have become semi-transparent and spread apart.

It should be borne in mind that if the heads are soaked in hot water the bunt spores will be killed and so rendered useless as a source of inoculum for smut experiments. Hot water should be used, therefore, only when a wetting agent is not available or when the spores are not required for inoculum.

[1] Contribution No. 897, Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

In one experiment in which 20 varieties of spring wheat (15 of *Triticum vulgare* and 5 of *T. durum*) were inoculated separately with *Tilletia foetida* and *T. caries*, a total of 68,000 heads were examined for bunt infection. When the heads were examined in the dry condition, 10,147 (14.9 per cent) were classified as infected. When examined after soaking in water infection was found to be present in 12,558 heads (18.5 per cent). From these results it will be apparent that an examination of the heads after soaking may be expected to give more accurate bunt counts.

In certain varieties it was frequently found that only one or two kernels in a head had become infected, and occasionally infection was restricted to
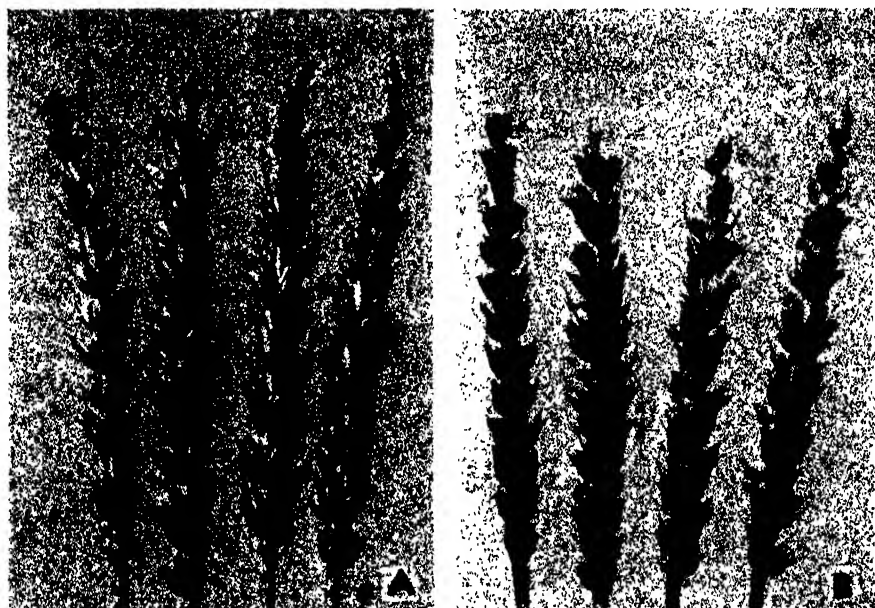
FIG. 1.   The effect of water-soaking of wheat heads on the visibility of bunt infection.   A. Smutted heads of Marquis wheat showing practically no symptoms of bunt before soaking in water with the exception of the head on the extreme left.   B. Same heads as in A after soaking for 24 hours in water containing a wetting agent.   Note darkening effect caused by bunt balls showing through water-soaked glumes.

only a portion of the kernel. In such instances it was extremely difficult to detect the infection by a visual inspection of the dry heads whereas, after soaking, the infected kernels were, in most instances, readily apparent. Partially bunted heads were encountered frequently in the variety Regent following inoculation with certain physiologic races of bunt. In one experiment necessitating the examination of 3,400 heads of this variety, bunt infection could be detected in only 34 (1.0 per cent) when the heads, but not the individual spikelets, were examined in the dry condition. After soaking in water, 100 (2.9 per cent) of the 3,400 heads were found to be infected.

It is believed that the method just described will be found to be particularly useful where a large number of heads has to be examined for bunt

infection. In practice it has been found that as many as 2,500 heads of wheat can be examined by one person in an hour.—W. POPP, Dominion Laboratory of Plant Pathology, Winnipeg, Manitoba, Canada.

Advantages of Natural Media and Environments in the Culture of Fungi.—When a fungus is encountered which grows or fruits well in nature but only poorly or not at all on artificial media in the laboratory it has seemed logical to resort to a natural medium, and to a fluctuating environment where such factors as range and periodicity of temperature, of light intensity, and of relative humidity are varied. It would seem that the culture of fungi might be handled in general to greater advantage were more consideration given to how and under what conditions fungi develop in nature and to what they require in the way of substrates and environments.

An example of one of the natural media used effectively for both the isolation and sporulation of certain fungi is pea-straw agar prepared from water agar and chopped pea straw which has been sterilized by fumigation.[1] When *Ascochyta pinodes* or *A. pinodella* is cultured on this pea-straw agar the hyphae grow into the medium and develop an abundance of dark, characteristic chlamydospores about and on the pieces of straw distributed through it, while pycnidia may appear on the surface of the medium. Where field surveys are to be made plates of pea-straw agar are taken into the field and even the underground parts of plants may be placed directly on the medium after shaking off the free soil. Since very little aerial mycelium develops on this agar the Ascochyta species may be identified usually in a week's time by direct microscopic examination of the plates. *Verticillium albo-atrum* is readily cultured from diseased tissues on this medium, simply by whittling small pieces of the infected tissue on the pea-straw agar. No sterilization of the plant part is required nor do laboratory contaminants which usually over-run potato-dextrose-agar plates interfere seriously with the recovery of *Verticillium*. The Verticillium hyphae grow into the medium and produce micro-sclerotia abundantly about and on the bits of straw, while typical conidiophores and conidia appear on the surface of the agar. Species of such genera as *Phytophthora*, *Pythium*, and *Rhizoctonia* also develop submerged hyphal growth in a medium of this sort and are easily recovered in pure culture by hyphal-tip transfer of the submerged mycelium from the original tissue platings of diseased material. *Alternaria solani*, which sporulates poorly or not at all on potato-dextrose agar, sporulates abundantly within a week after this medium is flooded with inoculum. A species of *Gloeosporium* which also did not sporulate on the usual artificial media did so profusely on the pea-straw agar.

. It has been evident that the kind of organic matter used as a medium need not be that on which the fungus is found in nature. For example, perithecia of *Mycosphaerella pinodes* have been produced abundantly on the

---

[1] Hansen, H. N., and William C. Snyder. Gaseous sterilization of biological materials for use as culture media. Phytopath. 37: 369–371. 1947.

wheat straw in a wheat-straw agar (fumigated wheat straw plus water agar) under proper environmental conditions, almost to the exclusion of either aerial mycelium or pycnidia.

These are only two examples of many natural media which have proved useful. Above and below-ground parts of plants, and scale and other insects have been used effectively for the culture of fungi after fumigation and placement on or in water agar, depending upon the substrate preferred and the purpose for which the medium is to be used.

The influence of a natural environment on the development of fungi is illustrated by still other examples. *Centrospora acerina* sporulates profusely on agar media outside a north window but not at all inside this window. The same is true of the imperfect stage of *Botryosphaeria ribis* and of *Mycosphaerella brassicicola*. A similar experience has recently been reported by Houston and Oswald,[2] who found that *Helminthosporium gramineum* sporulated rapidly and abundantly in an outdoor environment but not indoors.

The importance of both light and temperature in relation to the sporulation of fungi is well known but the results obtained here indicate further that diurnal fluctuation in both of these factors influences favorably the normal fruiting of fungi. Even the lack of fluctuation in humidity results in abnormal completion of spore discharge in some fungi. Perithecia of *Hypomyces solani*, *Gibberella roseum*, and *Mycosphaerella pinodes*, for example, exude their ascospores in wet masses or tendrils during periods of continuously high humidity but eject them forcibly into the air when periods of low humidity alternate with periods of high humidity.

Besides these environmental influences are still other factors for consideration such as the long cyclic fluctuations in the seasonal environment, and the direction of this cycle, whether, for instance, the cycle is entering a period of longer days with shorter nights or vice versa. That even these fluctuations have a bearing on the growth and sporulation of fungi in culture is evident from the relative speed, profusion, and quality of fruiting of various fungi when grown at different times of the year.—WILLIAM C. SNYDER and H. N. HANSEN, Division of Plant Pathology, University of California, Berkeley.

Powdery Mildew on Cherry Fruit in Washington.—During the latter part of July, 1944, the writer's attention was called to a disease affecting sweet cherry fruits harvested from orchards located in the upper portion of Squillchuck Canyon, near Wenatchee, Washington. An examination of the diseased specimens revealed the presence of powdery mildew hyphae and conidia. In a subsequent visit to the infected orchards the perefct stage of the fungus was found and later identified as *Podosphaera oxyacanthae* (DC.) D By.

[2] Houston, B. R., and J. W. Oswald. The effect of light and temperature on conidium production by *Helminthosporium gramineum* in culture. Phytopath. **36**: 1049–1055. 1946.

Although powdery mildew for many years has been known to occur on the leaves and shoots of the cherry in various sections of the United States,[1,2] a review of the readily available literature has failed to uncover any mention of fruit invasion. In the vicinity of Wenatchee the disease occasionally has been observed on leaves and shoots, but here also no previous records of fruit infection have been found.

The symptoms of the disease on fruits of sweet cherry are rather striking, and are distinctly different from those of any other disease known to attack
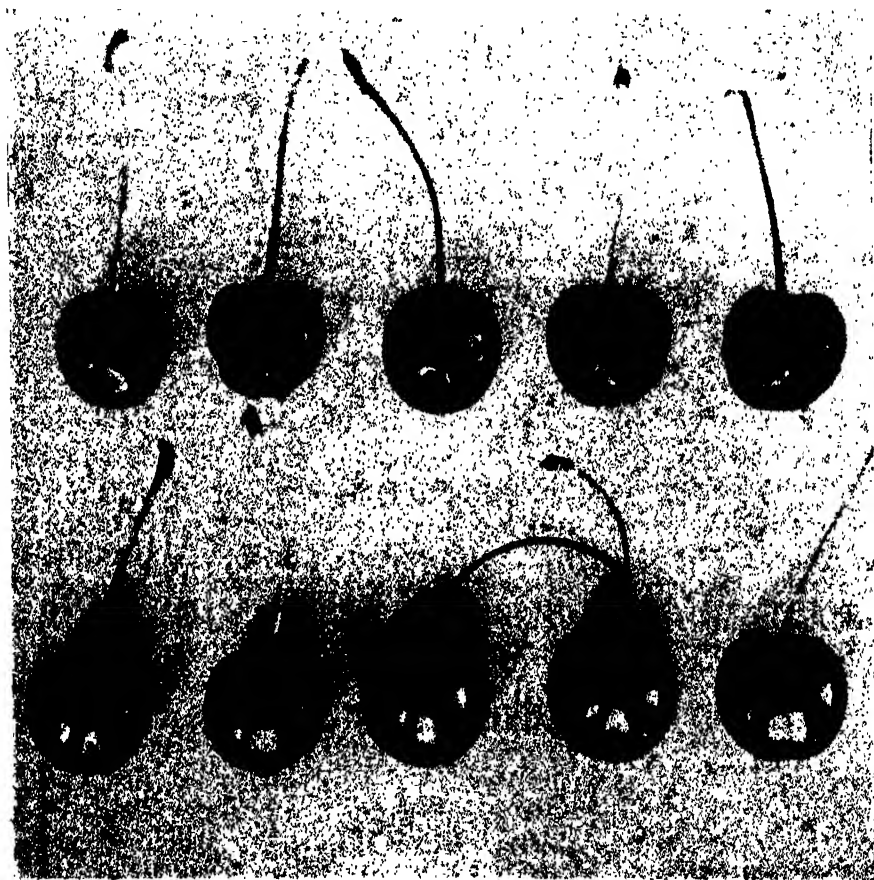
FIG. 1. Powdery mildew on fruits of the Bing cherry: Upper fruits diseased, lower ones healthy. Note the shriveled and dull appearance of the infected fruits as contrasted to the high gloss of the normal cherries.

cherries. The invaded areas are circular, or more frequently irregular, due to the coalescence of two or more infections (Fig. 1). A thin, white mantle of hyphae, which radiate from the center of young lesions, covers the infected

[1] Galloway, B. T. The powdery mildew of the cherry. U. S. Agr. Comm. Rept. 1888: 352-357. 1889.

[2] Stewart, Vern B. Some important leaf diseases of nursery stock. New York nell) Agr. Exp. Stn. Bul. 358. 1915.

portions and gives the fruit a decidedly dull appearance. Presumably as a result of excessive transpiration, the injured areas become somewhat depressed and the fruits definitely misshapen. The older lesions are reddish-brown and their skin texture is much tougher than that of healthy tissue.

In an attempt to analyze the environmental factors that had contributed to fruit infection, several diseased orchards were visited. They were located at an elevation 1200 to 1500 feet above those on the valley floor near Wenatchee, and the cherries generally mature 3 to 4 weeks later than those in the valley. During the evening of July 19, 1944, a heavy thunder shower occurred which was followed by hot, humid weather that lasted for approximately 1½ days. Prior to this shower, no fruit infection had been observed by any of the growers, but 2 days afterwards heavy infection was noticed in some of the orchards and in fruit being received at the packing house.

The trees in the orchard that was most severely affected were rather closely planted, and the tips of some heavily laden branches rested on the ground. It appeared that some of the trees might almost have functioned as giant moist chambers. Infection was so heavy on one of the trees that none of the cherries in the lower half were picked; with other trees lesser amounts of fruit were left because of the mildew. In the inner portions of the trees with heavy fruit infection there was invariably severe invasion of the succulent shoots and leaves. The presence of mature perithecia on some of the diseased leaves indicated that the initial attack had taken place rather early. Showers, interspersed with hot weather, during the middle of June and the first part of July probably promoted the initial leaf and shoot infection.

Another factor that possibly influenced the development of the disease in sweet cherries was the presence of mildew on wild choke-cherries (*Prunus demissa* (Nutt.) Walp.) growing in close proximity to the orchards. No significant morphologic differences could be found between the mildew on choke-cherries and that on sweet cherries. Tests to determine the pathogenicity of the choke-cherry mildew to sweet cherries have not been made. Growers report that frequently the choke-cherry is severely attacked by mildew when adjacent sweet cherries have little or no infection.

In the orchard that suffered greatest damage, the disease was far more severe on the Bing than on the Lambert or the Napoleon variety. In fact, several Lambert trees more or less surrounded by heavily infected Bing trees had only a trace of leaf infection and no mildew on the fruit. In an orchard at a slightly lower elevation in which picking largely had been completed, shoot infection was found on Bing, Black Tartarian, Lambert, and Napoleon varieties, and infected fruit was found on all varieties except Napoleon. Fruit inspectors reported that of the cherries examined by them in the packing houses, Black Tartarians appeared to be the most severely affected.

Local shippers inquired whether the mildew would continue to develop on the harvested fruit during storage and marketing, and whether the disease would predispose cherries to infection by decay fungi. To answer these

questions, 3 samples of cherries from a tree heavily infected with mildew were divided and stored at 40° F. and at 65° F., at a relative humidity of approximately 85 per cent. The samples were as follows: (1) All cherries infected with mildew, (2) no cherries visibly infected with mildew, (3) a composite of equal amounts of infected and visibly healthy fruits. A fourth sample similar to the latter was held in a moist chamber at 80° to 90° F. The cherries held at 65° or higher were examined after 5 days; those stored at 40° were examined after 10 days and again after 2 additional days at 65°. There was no evidence of an increase in size of the mildew lesions, nor were new infections found on any of the fruits that were visibly sound when stored. At 40° and at 65°, slightly more decay developed in mildewed than in sound fruit. However, since less than 4 per cent decay developed in the most severely affected sample, it appears that mildew does not markedly increase the susceptibility of the fruit to attack by decay fungi. Isolates obtained from rots centered at mildew lesions are arranged in decreasing order of prevalence, as follows: *Pullularia pullulans* (D By. and Loew) Berkh., *Botrytis cinerea* Fr., *Cladosporium sp.*, and *Rhizopus sp.*

Climatic conditions in the Wenatchee district during the summer of 1945 were entirely different from those in 1944, and the paucity of mildew in 1945 appeared to reflect these differences. During 1945 no precipitation was recorded at Wenatchee from June 9 to July 21, inclusive, and on July 22 only a trace of rain fell. Orchards that had had heavy mildew infection in 1944 were visited on July 6, 1945, but no sign of the disease was found on leaves, shoots, or fruits. Adjacent choke-cherries had slight mildew infection of shoots and leaves. A visit to the same orchards on July 27, as picking was being completed, revealed a trace of leaf, shoot, and fruit infection on Bing and Lambert varieties in one orchard. In another orchard, fruits on a sour cherry tree (Montmorency variety) had a trace of mildew, but no leaf or shoot infection could be found. The symptoms on sour cherry differed somewhat from those on sweet cherry in that the thin, almost indistinguishable mantle of fungus hyphae invariably radiated from the depression surrounding the stem.--HARLEY ENGLISH, formerly with the Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Wenatchee, Washington. Present address: Botany Department, Oregon State College, Corvallis, Oregon.

Hereditary Defects in the T.I. 448A Tobacco and Its Hybrids.[1]—In breeding for resistance to one disease, there is always the hazard of increased susceptibility to another. During the development of flue-cured strains of tobacco resistant to bacterial wilt,[2] a parasitic disease and two hereditary defects have been observed on T.I. 448A and its hybrids. These abnormalities increased the difficulties of producing wilt resistant strains suitable for

general culture, but it was possible by careful selection to isolate lines that were not affected by these troubles.

The occurrence of seedling blight associated with *Olpidium brassicae* (Wor.) Dang. (*Asterocystis radicis* de Wild.) was reported previously.[3] Two hybrid lines of T.I. 448A parentage were sown in a farmer's seed bed on unsterilized soil near Creedmoor, North Carolina.   One strain sown on
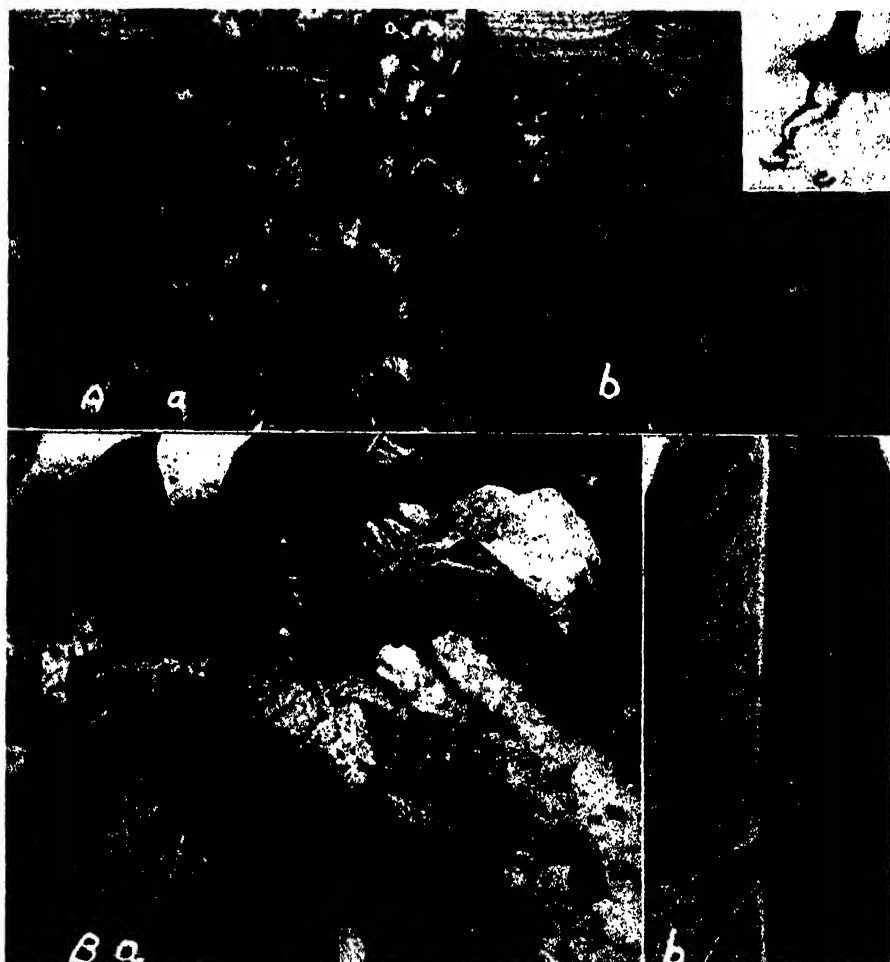
Fig. 1.   A. Resistant strain (a) and T.I. 448A (b) grown on steam-sterilized seed bed; (c) root system of T.I. 448A.   B. Leaf-spot-susceptible hybrid of T.I. 448A × 400 (a); vein necrosis (b).

20 square yards of bed grew slowly and over 90 per cent of the plants were killed in the 4 to 6 leaf stage by seedling blight.   Major symptoms were yellowing and withering associated with brownish root decay.   The disease was not observed on other wilt-resistant strains and standard varieties.

[3] Smith, T. E.   Seedling blight on tobacco in North Carolina.   U. S. Dept. Agr., Plant Dis. Reptr. 27: 273,   1943.

Another root disease occurred in seed beds at the Tobacco Branch Station, Oxford, North Carolina (Fig. 1, A). Major symptoms were stunted growth, brown root decay, and withering on hot days. This disease resembled seedling blight as well as ammonia toxicity. The cause is unknown but it was shown that neither *Olpidium* nor excessive rates of ammoniacal fertilizers was associated with it. All single-plant selections of T.I. 448A were susceptible. It was not possible to grow healthy seedlings on steam sterilized soil in beds outside or in the greenhouse. High soil temperatures reduced disease severity. Flue-cured varieties were resistant under all conditions. The F_1 of flue-cured varieties crossed with T.I. 448A grew normally, indicating that resistance was dominant. Susceptible F_3 and F_4 lines were found and eliminated.

An F_2 of T.I. 448A × flue-cured varieties showed 2.24 per cent of the plants with a leaf-spot disease in a population of 1518. White and brown necrotic lesions on the leaf blade combined with brown cankers of the primary and secondary veins were characteristic symptoms. Leaf breaking at the larger cankers on the primary veins also occurred. By selecting affected plants, uniformly susceptible lines were established. The cause is unknown. Washed tissue from young lesions was sterile on potato-dextrose agar and grafts failed to transmit the leaf spot. In crosses of susceptible and normal genotypes, the F_1 grew normally indicating that resistance was dominant. The F_2 from 3 such crosses averaged 10.86 per cent in a total population of 267 plants. The lesions on the secondary veins were similar to a "physiological weakness" reported as a recessive character by Johnson[4] in hybrids of Little Dutch and Cuban tobaccos.—T. E. SMITH, formerly stationed at the North Carolina Agricultural Experiment Station.

Barley Fertilizer and Seed Treatment Test.[1]—The 1941 barley crop of 49,954,000 bushels was the largest ever produced in Nebraska. By 1943 this production had dropped to 27,918,000 bushels. One of the major reasons for this drop was a severe attack of root rot, caused by unknown organisms. However, in view of the results obtained by several Canadian workers, preliminary fertilizer and seed-treatment tests seemed desirable in an effort to reduce the root rot damage. Greaney and Machacek[2] observed that the least amount of root rot of wheat caused by *Fusarium culmorum* (W. G. Swt.) Sacc. and *Helminthosporium sativum* P. K. and B. occurred in plots receiving a complete fertilizer. Russell and Sallans[3] found that phosphate fertilizers actually increased the amount of infection by the above mentioned organisms; however, the wheat receiving phosphate frequently outyielded

[4] Johnson, J. The inheritance of branching habit in tobacco. Genetics 4: 307–340. 1919.

[1] Published with the approval of the Director as Paper No. 418, Journal Series.

[2] Greaney, F. J., and J. E. Machacek. Progress report of the Dominion Botanist for the years 1931 to 1934, inclusive. Canada Dept. Agr., Div. Bot., 886 pp. 1935.

[3] Russell, R. C., and B. J. Sallans. The effect of phosphatic fertilizers on common root rot. Scient. Agr. 21: 44–51. 1940.

the non-fertilized plots in spite of the increased infection. Vanterpool[4] concludes that young wheat seedlings may be predisposed to fungal attack by the browning root-rot organisms at a critical stage in their development because of improper balance of available phosphorus and nitrate nitrogen in the soil. In later work,[5, 6] he reports that the addition of phosphate fertilizers reduced the damage from browning root rot and improved the growth of wheat seedlings.

Tests combining fertilizers and seed treatment were planted in 1945 in Merrick County in a fine sandy loam soil of low fertility and in 1946 in Hall and Buffalo Counties on sandy loam soils of moderate fertility. Table 1 indicates the nitrogen-phosphorus-potassium ratios used in 1945, and table 2 indicates the higher rates used in 1946. Nitrogen was supplied as Uramon, phosphorus as 45 per cent superphosphate.

TABLE 1.—*Barley fertilizer and seed treatment test planted April 6, 1945, in Merrick County, Nebraska*

| Treatment | Number of plants[a] | | Number of stems[a] | Yield[b] bushels | Test weight |
	May 2	May 17	June 6	per acre	(lb.)
None	39.4	34.5	52.9	11.5	41.0
0–30–0	37.0	33.7	65.1	14.1	41.0
20–0–0	41.5	36.0	75.2	15.4	42.0
20–30–0	35.6	32.5	82.0	19.2	42.0
New Improved Ceresan	45.6	39.0	58.5	12.3	41.0
0–30–0 plus NIC	40.6	33.7	56.7	12.2	39.0
20–0–0 plus NIC	41.9	36.1	71.2	15.3	41.0
20–30–0 plus NIC	38.0	33.6	90.7	24.0	43.0
Av. of non-treated-seed plots	38.4	34.2	68.8	15.1	
Av. of treated-seed plots	41.5	35.6	69.3	15.9	

[a] Average of two 5-foot lengths of row in each of four replications.
[b] Difference of 4.5 bushels required for significance.

Four replications of each fertilizer treatment were planted with certified Spartan barley, treated with New Improved Ceresan at the rate of $\frac{1}{2}$ ounce per bushel, and four replications of each fertilizer treatment were planted with untreated seed. Plots were 4 feet wide and 100 feet long. The quadrat method of harvesting was used for yield determinations. Five units of one square yard each were taken from each replication.

In 1945, seed treatment produced no significant benefits until used in combination with the fertilizer treatment containing both nitrogen and phosphorus (Table 1). This fertilizer gave a significant increase in yield over the check when used without seed treatment. However, combined with seed treatment it gave even more significant results. The latter plot could be

[4] Vanterpool, T. C. Studies on browning root rot of cereals. III. Phosphorus-nitrogen relations of infested fields. IV. Effects of fertilizer amendments. V. Preliminary plant analysis. Can. Jour. Res. 13: 220–250. 1935.

[5] ————. Studies on browning root rot of cereals. VI. Further contributions on the effects of various soil amendments on the incidence of the disease in wheat. Can. Jour. Res. (C.) 28: 240–257. 1940.

[6] ————. Pythium root rot of grasses. Scient. Agr. 22: 674–687. 1942.

detected throughout the experiment by its greener color and more vigorous growth, whereas none of the other plots could be readily identified by observation.

Increase in yield from seed treatment was not obtained in the 1946 test (Table 2). Nitrogen produced significant yield increases in all plots, when used alone and in combination with phosphate, irrespective of seed treatment.

No significant stand differences were obtained in any of the tests as a result of seed treatment or the application of fertilizers. There were differences in the number of stems per 5-foot length of row as recorded in table 1. These differences appeared to be associated with the differences in yields obtained with the various treatments.

The failure to obtain benefits from seed treatment in the Buffalo and

TABLE 2.—*Barley fertilizer and seed treatment test planted April 1, 1946*

Treatment	Number of plants[a]		Yield[b] bushels per acre Buffalo Co.	Test weight (lb.)
	Hall Co. April 22	Buffalo Co. April 22		
None	56	66	26.0	46.0
0–40–0	54	62	29.2	46.5
40–0–0	57	59	34.7	44.5
40–40–0	51	55	42.1	45.5
New Improved Ceresan	53	62	27.4	47.5
0–40–0 plus NIC	52	60	30.1	47.0
40–0–0 plus NIC	47	54	37.2	45.5
40–40–0 plus NIC	50	58	42.5	47.0
Av. of non-treated-seed plots	54.5	60.5	33.0	
Av. of treated-seed plots	50.5	58.5	34.3	

[a] Average of two 5-foot lengths of row in each of four replications.
[b] Difference of 4.2 bushels required for significance.

Hall County tests is not readily explainable. The soil was much higher in fertility than that represented in the 1945 test; and 1946 was also a much better year for barley production in Nebraska than was 1945. This may have been at least partially due to more favorable environmental conditions for the development of root rots in 1945. However, the results show that in certain years losses from root rot may be reduced by a combination of fertilizers and seed treatment. Although the damage from root rot fluctuates from year to year, the value of seed treatment from the standpoint of smut control alone makes seed treatment a practice to be highly recommended. When combined with fertilizer applications, it may become even more beneficial by reducing the damage from root rots.—J. E. LIVINGSTON, Nebraska Agricultural Experiment Station, Lincoln, Nebraska.

Comparison of Benzene Vapor with Certain Sprays in the Control of Downy Mildew of Cauliflower.[1]—In the United States, cauliflower (*Brassica*

oleracea var. *botrytis* L.) has been observed and reported[2, 3, 4] to be as susceptible as cabbage (*Brassica oleracea* var. *capitata* L.) to the downy mildew disease (*Peronospora parasitica* (Fr.) Tul.). Eddins[2, 3] has recommended Spergon (tetrachloro-para benzoquinone) and Fermate (ferric dimethyl dithiocarbamate) used as a spray or dust for the control of downy mildew on cabbage, under Florida conditions. Experimental data concerning control studies in relation to downy mildew on cabbage have shown that benzene when properly used will control downy mildew without apparent injury; certain sprays have also given satisfactory control. A preliminary report[5] dealing with benzene control of downy mildew on cabbage has been published.

The purpose of this paper is to show the contrast of the toxic effect of benzene vapor as compared with the beneficial effect of two organic sprays, Spergon (wettable) and Dow Seed Protectant No. 5 (tetrachloro-para benzoquinone) when used to control the downy mildew fungus on cauliflower.

In preliminary studies during the 1944–45 season benzene was used in a 66-square-yard bed to control downy mildew on cauliflower. Treatments at the rate of 50 cc. per sq. yd. on 5 successive nights per week under a wet cover of 48×44 thread count were started in advance of sporulation. Under severe mildew conditions this rate of treatment had effectively controlled downy mildew on cabbage. The results on cauliflower were unsatisfactory because benzene vapor failed to effectively control mildew sporulation and resulted in the death of many plants. The surviving cauliflower plants were stunted and developed slowly.

During the following seedbed season, 1945–46, benzene and the two organic sprays were compared in relation to effective control of downy mildew on cauliflower. Two cauliflower beds were sown on October 30, 1945; the same rate of seeding was used for both beds. One 10-yard bed was treated with benzene, 50 cc. per sq. yd. under a wet cover of 48×44 thread count. A total of 45 benzene treatments were applied, the first on November 8, 1945, and the final treatment on January 11, 1946. Treatments were started in advance of sporulation resulting from natural infection. The second bed (approximately 50 square yards) was divided into three sections; the center area remained the untreated control. The remaining two sections were sprayed bi-weekly with Spergon (wettable), 4 lb. per 100 gallons, and Dow Seed Protectant No. 5, 2 lb. per 100 gal., respectively. Orvus, at the rate of $\frac{1}{4}$ lb. per 100 gal., was used as an emulsifier for both sprays. A total of 12 sprays was applied, the first on November 9, 1945, and the final spray on December 31, 1945. A few spray applications were necessarily omitted because of rain.

2 Eddins, A. H.   Control of downy mildew with Spergon and Fermate.   Fla. Agr. Expt. Sta. Press Bull. 589.   1943.
3 Eddins, A. H.   Protecting cabbage plant beds from downy mildew with Spergon. Proc. Fla. Hort. Soc.   1944.
4 Felton, Mathias W., and J. C. Walker.   Environal factors affecting downy mildew of cabbage.   Jour. Agr. Res. [U.S.] 72: 69–81.   1946.
5 Foster, H. H., and J. A. Pinckard.   Control of cabbage mildew by means of benzene vapor.   (Abstr.) Phytopath. 34: 1000.   1944.

In these experiments Spergon (wettable) gave the best control, with a disease rating of 1 and average weight of 100 plant tops of 765 grams. Dow Seed Protectant No. 5 gave the second best control, with 1.5 for disease rating and 442 grams for weight of 100 plant tops. The benzene treatment was very unsatisfactory, resulting in the highest disease rating of 4 and causing severe plant injury resulting in an average weight of 99 grams for 100 plant tops. The untreated control plants gave a disease rating of 3 and a weight of 403 grams for 100 plant tops. Data are in table 1.

TABLE 1.—*Comparison of benzene vapor and certain sprays for the control of downy mildew on cauliflower*

Treatment	Wt. of 100 plants (cut at soil line)	Average weight	Average disease rating[b]
	Grams		
Spergon (wettable),[a]	811		
bi-weekly	719		
Dow Seed Protectant No. 5,[a]	410		
bi-weekly	475	442	1.5
Benzene, 50 cc. per sq. yd.,	103		
5 successive nights per week	96	99	4
Untreated control	450		
	355	403	3

[a] Fungicides furnished without cost through the courtesy of United States Rubber Company, Naugatuck Chemical Division; and The Dow Chemical Company.

[b] Grading system for cauliflower plants of size for field transplanting.

0 = Vigorous growth, no sign of infection.

1 = Vigorous growth, no active fungus sporulation, 1 or 2 lower true leaves may occasionally show trace or slight necrosis. All true leaves holding well, stems bright.

2 = Rather vigorous growth with occasional lower true leaf showing necrotic lesions and with some chlorosis—occasional lower leaf may be ready to drop or have dropped, usually no active fungus sporulation, little or no kill of plants in early seedling stage, stems bright.

3 = Only fair to poor growth with more or less stunting observed, usually slight kill of plants during early seedling stage. Active fungus sporulation may or may not be present. Necrosis mostly general on true leaves, with one or more lower leaves usually dropped. Frequently more or less stem discoloration.

4 = Marked stunting, usually considerable kill of plants in early seedling stage. Active fungus sporulation may or may not be present. Most plants showing abundant necrosis on true leaves, lower leaves usually dropped. Plants frequently weak and with stem discoloration. Secondary rots may be present on stems and roots.

It is evident that benzene is unsatisfactory for the control of downy mildew of cauliflower. At the rate used and under the conditions of this experiment benzene was exceedingly toxic to cauliflower. The death of cauliflower plants appeared to have been due, in large part, to the toxic effect of benzene vapor. Cabbage plants, grown in an adjoining seedbed and treated with the same rate of benzene, remained vigorous and relatively free from mildew.

It is interesting that two Cruciferae (cauliflower and cabbage) react so differently to the benzene vapor treatment. Both cauliflower and cabbage can be protected from downy mildew by Spergon (wettable) and Dow Seed Protectant No. 5. There was some indication in this experiment that Spergon stimulated cauliflower plants to increased growth.—H. H. FOSTER, Mississippi Truck Crops Branch Experiment Station, Crystal Springs, Mississippi.

REPORT OF THE 1947 ANNUAL MEETING OF THE SOUTHERN DIVISION, THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 1947 Annual Meeting of the Southern Division of The American Phytopatho logical Society was held in part as a section at the meeting of the Association of Southern Agricultural Workers, January 15, 16, 17, in Biloxi, Mississippi. A joint session was held with the Crops Division. Over one hundred plant pathologists were in attendance and more than twenty formal papers were presented.

The Report of the Committee on Internal Cork of Sweet Potatoes was adopted with a recommendation that it be made a part of the Proceedings of the Biloxi meetings and printed with the minutes in Phytopathology. It was also adopted that a copy of the report be forwarded to the Director of the Agricultural Research Administration, U. S. Department of Agriculture. The Report follows:

A temporary committee composed of L. H. Person, T. T. Ayres, H. H. Foster, Coyt Wilson, James Epps, E. R. Stamper, C. E. Steinbauer, and C. J. Nusbaum met at the Hotel Biloxi on January 16 to discuss policy and program for the internal cork disease of sweet potato.

This committee made the following recommendations:

1. That, because of the regional scope of the problem, an official committee on internal cork of sweet potato be established by the Southern Division of The American Phytopathological Society.

2. That D. E. Ellis, J. H. Miller, and C. J. Nusbaum be proposed as members of this committee to serve until the next work conference is held.

3. That the committee take immediate steps to initiate surveys in the sweet-potato-growing states of the Southern region in order to determine the occurrence and severity of the disease, to propose uniform sampling techniques, to prepare uniform data cards for the inspecting agencies and to request the services of the U. S. Department of Agriculture in assembling, compiling, and disseminating the results of the surveys.

4. That each State make the best possible use of its existing facilities in conducting the survey and that inspectors be adequately instructed in the sampling technique and methods of taking data.

5. That the committee make a study of certification regulations as they apply to internal cork infection of sweet potato seed stock and plants and that, meanwhile, each State take whatever steps deemed necessary in regard to restrictions designed to prevent the importation or intra-state movement of cork-infected planting stocks.

6. That plans for the initiation of collaborative research be deferred until further information on the occurrence of the disease is available.

7. That, if any state research agency contemplates undertaking a research project on internal cork of sweet potato the committee suggest that the project leader submit an outline of the project to the committee on internal cork in order that complete coordination of effort may be achieved.

C. J. NUSBAUM, *Chairman*

A business session was held on the morning of January 16 when the following officers were elected: *President*, A. L. SMITH; *Vice President*, C. J. NUSBAUM; *Secretary-Treasurer*, W. W. RAY; *Councilor*, S. G. LEHMAN.

Titles and abstracts of papers presented follow.

I. L. FORBES, *Secretary-Treasurer*

Improving Wilt Resistance and Yield of Cotton by Roguing and Selection. COTTON, JOHN R. Most wilt resistant strains of cotton have been developed from individual plant selections. The author has approached the problem from a different angle. Starting with a strain of low resistance and subjecting it to severe wilt conditions, by roguing and selection a highly resistant strain was developed having improved agronomic characters. The strain was a progeny from a D.&P.L.-Dixie Triumph cross. Bulk seed were planted in 1941 and roguing was done at 10-day intervals throughout the season. Only plants showing marked symptoms were removed, but the last roguing was made on the evidence of vascular discoloration. Some 300 plants were picked and studied in the laboratory for lint characters. From these, 100 plants were selected for planting in 1942. The same procedure was followed as in 1941. Only 76 selections were planted in 1943 and the same procedure was followed as in previous years. From the increase blocks in 1944, four

strains were selected for increase and testing. In a test at Baton Rouge in 1946 progeny No. 47-3-7-9 ranked first, having a lint percentage of 37.7, boll size of 85 bolls per pound, and lint length of 1 inch. Progeny No. 33-2-9-10 ranked second, having a lint percentage of 39.7, boll size of 80 bolls per pound, and lint length of 1¹⁄₁₆ inch. Both strains had better than 98 per cent wilt resistance. This was in comparison to the original strain, which had a lint percentage of 35.9, boll size of 88 bolls per pound, and lint length of 1 inch and only 35 per cent resistance.

A New Wilt-Resistant Watermelon. EPPS, JAMES M. Fusarium wilt of watermelon causes losses in Tennessee ranging from a trace to as much as 100 per cent. Most varieties now grown are not resistant to wilt. In 1941 a breeding program was initiated and many crosses between resistant and nonresistant varieties have been made. Some have had high resistance. Some seed lots received from the Mississippi Station in 1942, most of which were open pollinated, when tested in greenhouse and field were highly resistant to wilt and possessed high quality. All lots were still segregating. Several of the most promising lots were planted in wilt infested soil and the resistant segregates self-pollinated. One lot, originating from a cross between Dixie Queen and Klondike and highly resistant in the greenhouse and field, has been self-pollinated and selections made for uniformity. In grower trials it has survived in wilt infested soil, where the previous year 100 per cent of a commercial variety died. This melon possesses high quality, has small white seed, is green with darker green stripes, is oblong, and has a tough rind. It is prolific and usually sets many fruits. Melons average about 25 lb., with some weighing as much as 40 lb. Grower trials have indicated that the melon is satisfactory from the standpoint of size, shape, quality, resistance, and color. It is being released as a variety and has been named Miles, honoring the late Dr. L. E. Miles, who initiated the work in Mississippi in 1935. The Tennessee and Mississippi Stations have cooperated in the watermelon work.

Reaction of Species and Varieties of Cruciferae to Artificial Inoculation of Cabbage Downy Mildew. FOSTER, H. H. In greenhouse and plant beds during the past three years approximately 100 commercial varieties representing several species of Cruciferae were tested for resistance to *Peronospora parasitica* in repeated experiments. Plants were artificially inoculated, and kept under conditions favorable for sporulation. Infection was measured by a scale from 0 to 4. Under the conditions of these experiments all of the 60 commercial cabbage varieties tested were susceptible, falling in class 4 and occasionally class 3. All broccoli, Brussels sprouts, cauliflower, collard, and kohlrabi varieties tested were susceptible, class 4 or 3. All kale varieties tested, with one exception, fell in class 4. Siberian kale was resistant, falling in class 1, showing only slight necrosis. Rutabaga varieties varied from susceptible to tolerant. Four rutabaga varieties were tolerant, class 1 to 2, and one was susceptible, class 2 to 3. Turnip varieties tested varied from resistant to tolerant, in classes 0 to 2. Mustard varieties, with two exceptions, were highly resistant, classes 0 to 1; spinach and Chinese Broadleaf mustard varieties were less resistant. All radish and Chinese cabbage varieties tested were highly resistant, occurring in classes 0 to 1 with occasional slight necrosis on cotyledons but without sporulation. No variety tested was immune.

Stem-end Rot Fungi Attack Immature Citrus Fruit. HILDEBRAND, E. M. Stem-end rot fungi (*Phomopsis citri* and *Diplodia natalensis*) take a heavy toll of the Florida citrus (orange, grapefruit, tangerine) crop each year. Ordinarily infections develop only after mature fruit has been removed from the tree. As the result of this study, it appears that practically all developmental stages of citrus fruit from shortly after the blossom phase until maturity are naturally inoculated and become infected upon removal from the tree. The percentage of excised, immature fruits developing symptoms within one month from picking increased with the season from approximately 20 to 70 per cent between June and October. First symptoms developed on occasional fruits within a week. Others were still developing symptoms 16 weeks after removal. Histological studies of the button region of fruits of all ages frequently demonstrated the presence of stem-end rot mycelia or spores around, on, and under the calyx lobe. Since these mycelia were never detected below the cork layer on attached fruits, true penetration had not occurred. After fruits were picked, the fungus mycelia in the outer button tissues soon penetrated into the deeper button tissues and thence into the fruit. These studies support and extend those of Nadel (1944) on *Diplodia natalensis* in Palestine.

Effect of Presoaking Unshelled Peanuts on Fungus Control, Germination, and Emergence. IVANOFF, S. S. Soaking unshelled peanuts in water reduced fungus growth on the pods, decreased seed and seedling infection, and increased emergence in the field. In eight laboratory trials one lot of Spanish peanuts was soaked in water for 20 to 24 hours

and germinated in Petri dishes at 100 per cent humidity. A second lot (checks) of unsoaked peanuts was placed in Petri dishes at the time the first lot was put to soak. Records after 10 days showed 58 per cent of soaked pods with fungus growth and 96 per cent for the unsoaked pods. Species of *Fusarium*, *Penicillium*, and *Rhizopus*, *Sclerotium rolfsii*, and other fungi were present. In every trial the presoaked lot had fewer infected pods than the unsoaked lot. Average germination for the two treatments was 69 and 26 per cent, respectively. In four field trials emergence varied from no significant difference to great differences between treatments. In wet soil following heavy rains (2 trials) germination was about 35 per cent for both treatments. Shelled peanuts with and without Arasan treatment, showed no higher germination. In moist sandy soil, presoaked pods showed 77 per cent emergence for each of two trials, against 19 and 45 per cent for the unsoaked pods.

A Virus-Induced Top Necrosis in Bean. LeBeau, F. J. A virus producing a severe systemic necrosis of bean, *Phaseolus vulgaris*, was isolated from bean pods from Crystal Springs, Mississippi, in September, 1944. Mechanical inoculation induced in bean seedlings a fine systemic chlorotic stippling which rapidly became necrotic, and was followed by early abscission of leaves and blossoms. Certain bean varieties were killed outright. In other varieties (Black Valentine, Bountiful, Tendergreen) the disease was not usually lethal and very young seedlings frequently recovered. Abundant proliferation of leaf buds of recovering plants produced a bunchy growth of mottled and malformed leaves and occasionally blossoms. Other susceptible legumes included *Phaseolus lunatus* var. Small White, *P. multiflora*, *Vicia sativa*, *Pisum sativum*, *Pisum arvense*, *Vigna sinensis*, *Soja max*, *Lupinus albus*, *Cyamopsis psoraloides*, *Melilotus indica*, *Crotalaria intermedia*, and *C. spectabilis*. *P. lunatus* var. Fordhook was susceptible only to local infection. In tobacco, local chlorotic ring spotting developed and occasionally systemic ring spotting. Infection in cucumber produced a fine chlorotic spotting and severe stunting. Infectivity of the virus was lost after infective sap was heated at 68° C. for 10 minutes. Striking similarities in the symptomology on several hosts and in the thermal inactivation points suggested that the bean virus was probably related to the soybean bud-blight virus and the guar virus reported by Chester and Cooper.

Powdery Mildew of Soybean. Lehman, S. G. Powdery mildew has on several occasions been reported on soybean in Europe and America. The causal fungus when named has been assigned to the genus *Erysiphe*. In 1936 and 1944, powdery mildew was found in the pathology greenhouse at Raleigh, N. Carolina. Perithecia present were of the genus *Microsphaera*. In 1944, powdery mildew was collected by Prince in Western North Carolina and reported as *Erysiphe polygoni*. When these specimens were given more careful examination, perithecia of the genus *Microsphaera* were found. In September, 1945, *Microsphaera* was found on soybeans at several locations in Eastern North Carolina. The disease was present in the same areas in 1946. The fungus produces short, club-shaped conidiophores. Perithecia are dark brown to black, sub-spherical, and have an average of about 20 appendages. Appendages are two to three times as long as the diameter of the perithecium, and are three to five times dichotomously branched. In field and greenhouse inoculations the following varieties were susceptible: Arnredo, Cherokee, Herman, Ogden, Ralsoy, Seminole, Tokio, and a number of hybrid selections. Biloxi, CNS, Haberlandt, Roanoke, Rokusun, S–100, Volstate, and Woods Yellow, and several hybrid selections have remained free of mildew. (North Carolina Agricultural Experiment Station and Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Engineering, U.S.D.A., Cooperating.)

Dithane on Cotton Root Rot in Field Trial. Moore, Elizabeth J. Dithane (disodium-ethylene-bisdithiocarbamate) manufactured by Rohm and Haas was used in two forms, crystalline Dithane A–10 and a solution Dithane 14, in a field trial in an area heavily infested with the cotton root-rot organism, *Phymatotrichum omnivorum*. A Latin square of four treatments and a control replicated four times was designed. Dead plants were counted at regular intervals during the season. At the end of the summer, root systems were selected from each treatment for laboratory tests to determine whether the roots had absorbed any of this toxic material from the soil.

A Possible Mosaic Disease of Cotton Observed in Louisiana in 1946. Neal, D. C. What may be a virus or mosaic disease of cotton was observed at the Louisiana Agricultural Experiment Station on August 30, in plants of the Mebane variety obtained originally from Texas in 1944 and subsequently grown in the greenhouse and field in 1945 and 1946. Noticeable symptoms are the following: loss of chlorophyll in whitish bands between the leaf veins, reduction in leaf size, lobed margins, and an unusual stiff appearance of affected plants. In general, the mosaic pattern is easily observed from a distance

in the field. On September 5, five additional Mebane plants in another field had varying degrees of the malady. Two plants were seriously affected—the upper and middle branches were malformed and sterile—but in each case normal branches with mature bolls were observed on one side near the base of the plants. This feature, that is, plants having both diseased and normal branches, was frequently encountered. Other plants also were discovered with only the terminal leaves involved, indicating that the infection may have been of recent origin. In later inspections, the disease was found in a nearby area on two other unrelated lines, namely, a Dixie-Triumph × Deltapine hybrid (36–25) and a selection of Stoneville 2–B. The fact that the disease occurs in separate lines would indicate that a virus may be the causative agent of this abnormality; and that it is not a deterioration due to genetic causes. Preliminary attempts to induce the disease by inoculating plants with sap extracted from leaves of affected plants have thus far been unsuccessful.

Observations in 1945 and 1946 on the Relationship of Rainfall in Louisiana to the Incidence of Fusarium Wilt of Cotton. NEAL, D. C. In 1945 and 1946 striking differences in rainfall and the prevalence of Fusarium wilt of cotton were noted at Baton Rouge, Louisiana. The rainfall in 1945—May through August—exceeded slightly the 20-year average, with higher departures occurring only in June and August. In 1946, it greatly exceeded this average, the departures being much higher for May and July and slightly higher for June. Maximum temperature ranges of 90° to 97° F. occurred each year during these months. In 1945 wilt appeared early, June 12, and became progressively worse as the season advanced; and crop failures resulted for several susceptible varieties. In 1946, even with the more susceptible varieties, only a scattering amount of wilt developed before the middle of August. The rainfall and bi-weekly wilt infection data indicate that the incidence of the disease is favored by alternately wet and dry periods during the optimum period for infection, June to August, and by rainfall slightly in excess of the 20-year average. In contrast, an extended period of excessive precipitation at this time is unfavorable for the disease.

Studies of Boron Deficiency in Sweet Potatoes. NUSBAUM, C. J. The influence of different rates of application of B at high and low levels of N, P, K, and Ca upon the incidence of B-deficiency, growth cracking, yield and quality of sweet potatoes was studied in a replicated field experiment. Variations of 3–9–9 fertilizer, which is the standard formula for sweet potatoes in South Carolina, included 0–9–9, 6–9–9, 3–9–0, 3–0–9, and 3–0–0, applied at the rate of 1,000 lb. per acre both with and without added lime. Borax was supplied at rates of 0, 5, and 20 lb. per acre. Without added borax, B-deficiency was severe with the low P (3–0–9), standard (3–9–9), and high N (6–9–9) fertilizers and was slight with the low N (0–9–9), low K (3–9–0), and low PK (3–0–0) fertilizers. Lime tended to increase B-deficiency. In all cases, B-deficiency symptoms were practically eliminated by an application of 5 lb. of borax per acre. Growth cracking was most severe with the low P (3–0–0), and high N (6–9–9) fertilizers and in most cases increased in proportion to the rate of applied borax. The highest yields and returns were obtained from the standard (3–9–9) fertilizer plus borax. With this fertilizer treatment, regardless of the Ca level, the returns from the 5 and 20 lb. applications of borax were about the same and generally exceeded the values for no borax by about $100.00 per acre.

Studies of Internal Cork, a Probable Virus Disease of Sweet Potato. NUSBAUM, C. J. Preliminary investigations of the nature and seasonal development of internal cork of sweet potato indicate that this disease is caused by a pathogenic virus. A constant association of a definite succession of foliage symptoms, indicative of a virus infection, with the internal cork symptom of the roots has been noted. Vein feathering and mottling of the leaves is followed by a reddish to purple splotching, sometimes in ring form, and then by a gradual fading out of leaf patterns. These leaf symptoms have been induced consistently in plants arising from healthy roots by making core grafts of corky root tissue into the healthy sweet potatoes. It has been shown that infected roots are able to carry the disease through storage and produce it in a succeeding crop grown from them. Although the disease appears to bring about premature deterioration of foliage, no noticeable reductions in yield have been noted. The corky areas in the flesh begin to appear about the time the first roots reach marketable size and these areas continue to increase in number and size in the field and to an even greater extent in storage. There has been considerable variation in the reaction of different varieties and strains of sweet potato, both as to the amount of corking of the roots and the character of leaf symptoms produced. Porto Rico and related strains have had considerable amounts of internal cork and Nancy Hall but little.

Results of Seed Treatment in Controlling Damping-Off of Cotton in Mississippi. PRESLEY, JOHN T. A study of the results of cotton seed treatments in Mississippi indi-

cates that no general statement will hold true for all conditions and soil types in the State. The unusually cool wet spring of 1946 was favorable for the development of damping-off and Thielaviopsis root rot of cotton seedlings. More rapid emergence and higher stand counts were generally obtained from acid delinted or reginned seed than from fuzzy seed, irrespective of treatment. All treated seed gave consistently better stands than nontreated seed. Dow 9 (zinc trichlorophenate) and DuBay 1452–F (7.7 per cent ethyl mercury p-toluene sulfonanilide) gave higher average stand counts than the remainder of the chemicals used. The loss from Thielaviopsis root rot this year was sufficient to warrant control measures, particularly for the Delta area of Mississippi. One of the more stable fungicides combined with the regular seed protectant may prove effective. (U. S. Department of Agriculture cooperating with Mississippi Agricultural Experiment Station.)

Cotton Seed Treatment Tests in Oklahoma in 1946. RAY, W. WINFIELD. Based on final seedling stands, no statistically significant difference between DuBay 1452–F (7.7 per cent ethyl mercury p-toluene sulfonanilide) and Dow 9–B (50 per cent zinc 2,4,5-trichlorophenate) was found when these two chemicals were applied to fuzzy-matted, reginned, reginned-matted, and acid-delinted seeds. All treatments were significantly superior to the nontreated fuzzy lot. The stands of seedlings from the greatest to the least were: acid-delinted, lightly reginned, heavily reginned, reginned-matted, fuzzy-matted, and fuzzy (nontreated). In the C–Test, involving 5 chemicals and 4 types of seeds (fuzzy, 2 degrees of reginned, and acid delinted), DuBay 1452–F produced the best stands. Dow 9–5 (15 per cent Dow 9 and 85 per cent chloranil) and a dust composed of paraformaldehyde, benzoic acid, and dimethylolurea gave the lowest stands, whereas lots treated with a mixture of Fermate-DuBay 1452–F (6 parts to 1) and with 5 per cent mercury trichloroethylene were intermediate.

Cotton Seed-Treatment Test. ROGERS, C. H. Of 24 differently treated seed lots, emergence counts varied from a low of 52 per cent for nontreated fuzzy seed to a maximum of 91 per cent for acid-delinted seed or acid-delinted seed plus fungicidal dust treatment. No benefits were derived from matting seed regardless of further treatment. Seed that were reginned once were somewhat superior in performance to seed that were reginned four times. No significant differences were obtained in yields of seed cotton.

Studies on the Seed Treatment of Rice. RYKER, T. C., and S. J. P. CHILTON. Replicated rod row and field seed treatment tests on rice were conducted during the seasons 1943–1946 inclusive. The single rod-row tests, comprising three materials, Arasan (tetramethyl-thiuram-disulfide), Spergon (tetrachloro-parabenzoquinone), and New Improved Semesan, Jr. (organic mercury dust) and three dates of seeding, March, April, and May, were made at the Rice Experiment Station, Crowley, Louisiana. Stand counts and yield data were obtained. Five field tests with Arasan were planted with the drill that the grower was using on the farms. Stand counts were obtained by counting five rows ten feet long of treated and nontreated seed in four places. Arasan gave the best results, increasing stands nearly 25 per cent in the March and April plantings and 10 per cent in the May planting. Small but consistent increases in yield occurred where seed was treated. Arasan gave consistently better germination in seed stored for eight months and materially reduced the stored grain insect infestation.

Cotton Seed-Treatment Studies in Georgia. SMITH, A. L. Three-year trials of new dust disinfectants indicate DuBay 1452–F (6.5 per cent ethyl mercuri-p-toluene sulfonanilide) and Dow 9B (50 per cent of the zinc salt of 2,4,5-trichlorophenate) are satisfactory for cotton seed. DuBay 1452–F is equally as effective as New Improved Ceresan (5 per cent ethyl mercuric phosphate), now most commonly used, and is less vesicant and less disagreeable to workers. Dow 9B while slightly less effective than New Improved Ceresan is much less toxic to animals, less vesicant, and less disagreeable to workers. Fewer shank lesions occurred on seedlings treated with Dow 9B than with other materials, which suggests greater protection in the soil. Reginned seed produce 3 to 5 per cent fewer seedlings than fuzzy seed. However, ease of planting, more rapid emergence and better emergence with low soil moisture result in grower demand and widespread use of reginned seed. (Cooperative studies with the Cotton Seedling Disease Committee.)

Wilt Resistance in Empire Cotton. SMITH, A. L., and W. W. BALLARD. Empire is a promising new variety of cotton now in the third year of commercial production. The original parent plant was re-selected in 1935 from a field planting of Stoneville 2 originally selected in 1931. Selfing was started in 1938 and a number of lines have been selfed continuously since that date. Early field tests on wilt soil with pooled progenies indicated only moderate resistance. Tests of selfed lines in 1944, 1945, and 1946 showed a

wide range in resistance from high susceptibility to resistance comparable to that of the best commercial varieties. The wide range in resistance indicates the heterozygosity of the original plant and suggests its probable hybrid origin between Stoneville 2 and a wilt-resistant variety. The uniformity of the selfed lines for agronomic characters facilitated an immediate shift in production to those having high wilt resistance.

The Effect of Soil Fumigation on Growth and Yield of Peach Trees. TAYLOR, A. L., and C. W. McBETH. Peach trees at Tifton, Georgia, planted in sites treated with chloropicrin to control root-knot nematodes (*Heterodera marioni*) had significantly larger trunk diameters after two years than those planted in untreated sites. Optimum size of site was apparently about 6 feet. Similar results were obtained where D–D was used instead of chloropicrin for nematode control. In another experiment peach trees in sites treated with chloropicrin and interplanted with cover crops susceptible to root knot (cowpeas and Austrian winter peas) produced 21.2 lb. of peaches per tree for their first crop, while trees in untreated sites with the same cover crops produced 4.8 lb. of peaches per tree. Trees in sites treated with chloropicrin and interplanted with cover crops resistant to root knot (*Crotalaria* and oats) averaged 41.9 lb. of peaches per tree, and trees in untreated sites with the same cover crop program produced 6.9 lb. of peaches.

White Rot of Shallot and Its Control. TIMS, E. C. White rot (*Sclerotium cepivorum* Berk.) occurs in the shallot-growing section of Southern Louisiana. The soil in a few fields is heavily infested with the fungus, which causes severe injury to shallots during the winter months. Small scale tests have been conducted over a period of three years to control the disease in the soil. A number of varieties and strains of shallot were tested for resistance. None of them showed any promise whatever. Lime was applied to infested soil in the field in sufficient amounts to change the pH from 5.6–5.8 to about 7. The amount and severity of infection was reduced to some extent, but satisfactory control was not obtained. Certain other chemicals applied in small amounts to the soil around the shallot plants gave promising results. Semesan (30 per cent hydroxymercurichlorophenol) at the rate of 1 oz. per gallon of water and mercuric chloride (1–500) applied at the rate of 80 cc. per plant gave almost complete control. A number of the common seed treatment compounds were used for dusting shallot seed sets before they were planted in diseased soil. None of them reduced the amount of white rot to any appreciable extent.

Symptoms Induced at Standardized Wounds by Fungi Isolated from Dry Turpentine Faces. TRUE, R. P., and MILTON M. SMUCKER. *Diplodia pinea* (Desm.) Kickx, *Gloeotulasnella pinicola* (Bres.) Rogers, and *Ceratostomella ips* Rumbold produced pitch soak and dry face (cessation of normal gum flow after wounding) at cambium-depth inoculated wounds in slash pine (*Pinus caribaea* Morelet). *Diplodia natalensis* Evans induced considerable pitch soak but little dry face. No notable symptoms resulted from inoculating wounds with species of *Cytospora*, *Trichoderma*, *Penicillium*, *Geotrichum*, *Schizophyllum*, *Stereum*, and seven undetermined fungi. The three dry-face fungi and possibly *Diplodia natalensis* are considered capable of invading uninfected turpentine faces. The other fungi tested are regarded as secondary invaders usually incapable of infecting healthy faces. Gum yield and pitch soak measurements showed that temporary gum flow stimulation and extensive pitch soak symptoms usually preceded the dry face condition. Symptoms were most pronounced following inoculations with *Diplodia pinea*. Wound response differences were largely attributable to the different fungi tested, but individual tree characteristics also determined in part the type and severity of symptoms. Present experimental evidence indicates that certain fungi may often have a primary rôle in inducing dry face, a major source of loss to the naval stores industry.

Relation of Environment to the Incidence of Fusarium Wilt of Cotton. YOUNG, V. H. One hundred ninety-two soil samples from eleven areas in Arkansas failed to show correlation between the pH of the soil and cotton wilt. The pH varied from 4.66 to 8.4. Wilt was severe on potash-deficient areas which are often acid, but acidity did not appear to be the governing factor. In certain areas cotton responded to applications of boron but the incidence of cotton wilt was not lowered by the use of fertilizers containing boron. Records at the Cotton Branch Experiment Station at Marianna, Arkansas, taken for eighteen years showed that high rainfall, especially prior to August, was generally correlated with higher incidence of Fusarium wilt of cotton whereas in drouth years or years with prolonged dry periods during June and July there was less wilt. In the 5-year period from 1929 to 1933 cotton was planted at 2-week intervals from April 15 to July 1. The 5-year average for April 15 plantings was 35 per cent wilted plants in contrast to 3 per cent for the July 1 plantings. High soil moisture during more of the growing season is believed to be the principal cause of higher wilt incidence in early planted cotton.

REPORT OF THE FOURTH ANNUAL MEETING OF THE POTOMAC DIVISION, THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The meeting was held on February 19 and 20, 1947, at the Plant Industry Station, Beltsville, Maryland. Some 75 members were in attendance. In addition to the 29 research papers presented, the program included the following: A survey of the results being obtained with the newer fungicides, H. P. Barss and committee; plans for more effective disease reporting, P. R. Miller and R. J. Haskell; the golden nematode situation in this country and in England, W. S. Pophan. Demonstrations were given of the slurry method of seed treatment, G. F. Miles, and of aerosol fumigation for greenhouses, F. F. Smith. A dinner, attended by 85 members and guests, was followed by an evening program of motion pictures provided through the courtesy of the Sherwin-Williams and the E. I. Du Pont Companies, and an illustrated lecture on Plant Virus Research in England and Ireland by Dr. R. H. Larson of the University of Wisconsin. Officers elected for 1947–1948 were V. F. TAPKE, *President;* W. F. JEFFERS, *Vice President;* W. W. DIEHL, *Secretary-Treasurer;* and E. E. CLAYTON, *Councilor.*

ABSTRACTS OF PAPERS

Black Rot as a Storage and Market Disease of Sweet Potatoes. COOLEY, J. S. The recent expansion of the marketing of freshly-dug, non-cured sweet potatoes and the practice of washing in preparation for marketing give new emphasis to the importance of investigations now in progress on the black-rot disease in the market. When black rot is present in the crop at digging time it will continue to develop during storage. It will even develop in non-cured roots during the relatively short time they are being marketed. Washing of non-cured sweet potatoes, with the unavoidable skinning, favors infection and spread of the disease. It is important that growers know how to handle this crop when black rot is present in it at digging time in such a way that the black-rot losses will be minimum. Considering the possibility of continued development of black rot, less loss will occur when the sweet potatoes are sold in an active nearby market as soon as dug. When such a market is not available it is preferable to cure for 10 days immediately after digging and grade out the rots that developed during curing; then market as soon thereafter as possible. A campaign for black-rot control by all States producing sweet potatoes should greatly reduce present heavy loss from this disease.

A Proliferating Gall on Blueberry Plants Caused by an Actinomyces. DEMAREE, J. B. A fungus tentatively identified as an *Actinomyces* causes a tumefaction often accompanied by bud proliferation at or near the primary node of blueberry (*Vaccinium*) plants. The aberrant growth often attains a huge size in comparison with that of the infected stem. The numerous buds emerging from the gall tissues usually become aborted early, or a few buds may differentiate into short fleshy shoots with abnormal leaves, or sometimes several buds develop into spindly shoots with small but normal-sized leaves, giving rise to a growth resembling a witches'-broom. The fungus readily infected young blueberry plants when bits of agar containing hyphae and spores were applied to injured tissues in the region of the primary node. It was reisolated from artificially infected plants and in turn was used successfully as inoculum. The fungus was originally isolated from naturally induced galls on young blueberry plants grown in soil composed partly of leaf mold taken from a forest floor where the huckleberry (*Gaylussacia baccata*) grew. Among several different lots of seedling blueberry plants of different parentage, totaling over 1,700, the incidence of natural infection per lot varied from 8 per cent to 70 per cent, an average of 26 per cent. Many of the huckleberry plants in the woodlot where the propagating soil was obtained have an undetermined witches'-broom disease.

Germination of Oospores of Pythium Butleri and Pythium tardicrescens. DRECHSLER, CHARLES. When transferred to a shallow layer of distilled water, oospores of *Pythium Butleri* from maizemeal-agar plate cultures 60 days old were found to germinate freely by the production of zoospores. Preliminary to such germination the massive oospore wall is commonly reduced to a thin outer layer through resorption of a thicker inner portion, while the protoplasm loses the organization characteristic of the resting condition to present an appearance much like that in the spherical sporangium of *P. debaryanum.* The granular contents, on being discharged into a vesicle at the tip of a simple or occasionally somewhat branched evacuation tube commonly 40 to 225 μ long and 2.5 to 5 μ wide, are transformed into approximately 10 motile zoospores. Oospores from a 112-day-old culture of *P. tardicrescens* that was isolated from a blackened root of *Bidens sp.* gath-

ered in Arlington, Va., Oct. 11, 1943, likewise germinated by producing about 10 zoospores in a vesicle at the slightly reflexed orifice of a simple or occasionally somewhat branched evacuation tube, which here commonly measured 60 to 350 μ in length and 2 to 3 μ in width.   After discharge the persisting outer layer of the oospore wall in each species sometimes encloses a separate sporangial envelope.

Some Effects of Spergon as a Pea Seed Treatment on Soil Fungi. HASSAN, H. H., and C. E. COX. The value of Spergon (tetrachloro-para-benzoquinone) seed treatment in improving stand, reducing damping-off and root rot of pea seedlings is known. Its effect on soil-inhabiting fungi apparently has not been investigated. The number of fungi which could be cultured from a sample of soil planted with Spergon-treated pea seed was less than half that from a similar sample of soil planted with untreated seed. When aliquots of soil were exposed to various concentrations of Spergon in soil suspension for varying periods of time before culturing, it was found that a 1–100 concentration inhibited all growth after momentary exposure. A 1–1000 concentration permitted some colonies to develop up to 24 hours, but with longer exposures prevented all growth. Weaker doses of Spergon prevented the growth of increasingly greater numbers of fungi with increasing exposure time. The pea pathogens, *Pythium sp., Rhizoctonia solani, Ascochyta pinodella,* and *Fusarium solani* f. *pisi* were cultured on Czapeck's agar medium containing various concentrations of Spergon. Concentrations of 1000 p.p.m. and 100 p.p.m. Spergon in the medium killed *Pythium sp.,* and reduced the growth rate of *A. pinodella* and *F. solani* f. *pisi.* Growth of *R. solani* was prevented on medium containing 1000 p.p.m. Spergon, but the fungus was not killed.

"Specificity" of the Metallic Dithiocarbamates in the Control of Certain Vegetable Diseases. HEUBERGER, J. W. Research conducted in Delaware (1943–1946, incl.) on the development of metallic dithiocarbamates as fungicides has concerned itself with a study of various metallic salts in the dimethyl series, a comparison of similar metallic salts in the dimethyl and ethylene series, and an evaluation of these materials for control of several vegetable diseases. Data obtained show the following: (1) in the dimethyl series, the zinc salt (Zerlate) was more effective than the sodium, calcium, iron (Fermate), copper, and lead salts for the control of early blight on tomato and potato, was more effective than the sodium, calcium, and iron salts for control of tomato anthracnose, and was more effective than the iron salt for control of downy mildew on cucumbers; (2) in the ethylene series, the zinc salt was more effective than the iron salt for control of early blight and late blight on potato and tomato, for tomato anthracnose, and for downy mildew on cucumbers; (3) the zinc and iron salts in the ethylene series were more effective than corresponding salts in the dimethyl series for control of early blight and late blight on potato and tomato and for downy mildew on cucumbers, but were slightly less effective against tomato anthracnose.

Evaluation of Several Fungicides in Preventing Infection of Sweet Potato Slices by Ceratostomella fimbriata. JEFFERS, W. F. On the basis of percentage active ingredient, a dilution series, of 100 g. per liter to 0.001 g. per liter, was prepared for each of several fungicides. Slices of Maryland Golden sweet potato were placed in an inoculum of the black-rot organism and then dipped in the fungicide solutions. These pieces were then placed in moist chambers and incubated at 28° C. for four days. By this time the surface of slices dipped in water was covered with growth of *Ceratostomella fimbriata* and there was heavy production of ascospores and conidia. Puratized N5-E (phenyl mercury triethanol ammonium lactate), Isothan Q-15 (lauryl isoquinolinium bromide), mercuric chloride, Semesan Bel (hydroxymercurinitrophenol) and Puratized 177 (para amino phenyl cadmium dilactate) showed high toxicity to the organism. Borax, wettable Spergon (tetrachloro-para-benzoquinone), Zerlate (zinc dimethyldithiocarbamate), and Phygon (2,3 dichloro 1,4 napthoquinone) were moderately effective. Copper 8 hydroxyquinolate, Fermate (ferric dimethyldithiocarbamate) and zinc ethylene bis dithiocarbamate were only slightly effective. Manganese ethylene bis dithiocarbamate and Tersan (tetramethylthiuramdisulfide) were of little value in preventing infection. In general the materials giving best control of the black-rot organism caused most injury to sweet potato tissue.

Diseases with Scab-like Symptoms Observed in Southern California in 1946. JEHLE, R. A., and ANNA E. JENKINS. The senior writer examined ornamentals in Southern California for diseases with scab-like lesions during November and December, 1946. No violet scab (*Sphaceloma violae*) was found. Diseases with similar symptoms were prevalent and destructive on other ornamentals, namely *Hedera helix* (English ivy), growing as a lawn cover in Los Angeles, Pasadena, Altadena, Fullerton, and Redlands; *Pelargonium peltatum* (ivyvine geranium), lawn plantings in the same localities, also in Santa Ana, and *Schinus molle* (California pepper tree), planted on streets in Los Angeles. *E.*

helix and *S. molle* were severely defoliated. Representative specimens were referred to the junior writer who reported: The disease of *H. helix* is called "scab of English ivy" (*Sphaceloma hederae* Bitancourt and Jenkins (1946)). That of *P. peltatum* requires further study for exact diagnosis. Its symptoms resemble Ringuelet's (1927) intumescences on *P. peltatum*, also those of the so-called "dropsy" or "oedema" of common *Pelargonium*, in one instance, at least, reported (F. C. Stewart, 1910) on *P. peltatum*. What is probably *Macrosporium pelargonii* Ell. & Ev., originally described on *Pelargonium sp.* from Pasadena, is present, although apparently nonpathogenic. Leaf lesions on *S. molle* are consistent with a *Sphaceloma* disease. In January, H. S. Fawcett sent specimens of all three diseases from other localities in Southern California.

Additional Records on Distribution of Plantain Scab. JEHLE, R. A., E. A. WALKER, D. A. PRESTON, and ANNA E. JENKINS. Jenkins and Bitancourt in 1946 described a new species, *Sphaceloma plantaginis*, causing plantain scab. This was based on herbarium material (oldest specimen collected at Still Pond, Md. 1891), and recent collections since 1939, mostly on *Plantago spp.* and *P. rugelii*, representing 62 collections from 14 States east of the Mississippi River and from the District of Columbia. A survey in Maryland during 1946 extends the known distribution of scab on *P. rugelii* to 36 localities (in 16 counties) of which 30 are new. One herbarium specimen was found showing scab dating back to 1905. Specimens collected from two States west of the Mississippi River, Minnesota and Oklahoma, extend the known distribution to sixteen States and the District of Columbia. Additional collections were reported from Virginia and West Virginia to make collections now available from 102 locations. In Maryland, plantain scab was very abundant on *P. rugelii*, being most severe on plants located along sidewalks, roadways, and parking lots. In open fields scab was less severe and more difficult to find. Two specimens of *P. lancelata* having scab-like lesions suggesting plantain scab were collected in Virginia and Maryland.

Periconia circinata (Mangin) Sacc. as a Possible Causal Factor in "Milo Disease." LEUKEL, R. W., and FLORA G. POLLACK. Isolations from the roots of Colby milo grown in soil from fields naturally infested with "milo disease," frequently yielded a fungus with a dark mycelial growth producing either abundant conidiospores or chains of thick-walled chlamydospores. The predisposing factors causing these variations in sporulation have not been extensively studied, but they seem to include the type and depth of the media used, the presence of contaminants, and other conditions of environment. This distinctive fungus was identified as *Periconia circinata* (Mang.) Sacc. Heavy inoculation with this fungus in steamed soil caused severe root injury in Dwarf Yellow and Colby milos, susceptible to the "milo disease," but had no apparent effect on milos resistant to "milo disease." In soil less heavily inoculated, susceptible milo plants matured, but were stunted, produced small heads or none at all, and died considerably in advance of milo plants resistant to "milo disease," and grown in the same soil. The susceptible plants resembled in many respects those grown in soil naturally infested with the "milo disease" and this fungus may be a contributing causal agent of that disease.

Effect of Temperature and Moisture on the Efficacy of Soil Fumigants. McCLELLAN, W. D., J. R. CHRISTIE, and NORMAN L. HORN. The effect of soil temperature and moisture on the efficacy of Larvacide (chloropicrin), D-D (dichloropropene and dichloropropane), Dowfume G (methyl bromide), and Dowfume W-15 (ethylene dibromide) was investigated by means of Wisconsin-type soil temperature tanks. Results were based on the effect of 2.5 ml. of each fumigant on three test organisms placed in the soil seven inches from the point of injection. The effect of Larvacide was most rapid at 98° F. It was only slightly effective against *Heterodera marioni* at lower temperatures. D-D was not effective against the fungi, but gave complete nematode kill after one day's exposure at 82° or 98° F., and after three days at 62° or 72°, but very little at 45° or 55° even after 13 days. Dowfume G was not effective against *Fusarium oxysporum f. callistephi*, only slightly effective against *H. marioni*, but had considerable effect against *Sclerotium rolfsii* at 72°, 82°, or 98°F. Dowfume W-15 was not effective against *F. oxysporum f. callistephi* but was effective against *S. rolfsii* at 72° F., and above after six days. Against *H. marioni* it was most effective at 98° F., and was effective at all temperatures after three days. In general, all four fumigants were most effective, and were retained in the soil for longer periods, when the soil was wet.

Survival of Labile Viruses in Desiccated Leaf Tissue. McKINNEY, H. H. Seven species of labile viruses have been desiccated over $CaCl_2$ at 1.5° C., and then stored in tight bottles without desiccant at 1.5° C. Atmospheric oxygen was not removed. All species showed activity for periods of 177 days and longer. Viruses of tobacco ring-spot, Southern celery mosaic, and cucumber mosaic were highly active after 547, 765, and 745 days, respectively. Viruses of potato "Y" mosaic and prairie wheat yellow mosaic were

moderately active after 420 and 701 days, respectively. When stored at 26° C., activity was lost or very low after two weeks. Tests with the tobacco ring-spot virus show slight survival after 13 weeks storage at 26° C., when desiccation was completed over $Mg(ClO_4)_2$ (anhydrone) at 1.5° C., and continued with the same desiccant during the storage period. It appears that the reactions favoring rapid inactivation of virus when stored at laboratory temperatures, are favored by extremely small quantities of water in the tissues. Complete desiccation should facilitate survival of these viruses during transportation, and during storage in the event of breakdown in a refrigeration system. Since the method greatly reduces the stocking of viruses in growing plants, and the chances of contamination, it should serve as a distinct aid in extending the type-culture method to labile plant viruses.

Wheats Resistant to and Immune from Mosaic in Nursery Tests Are Susceptible When Inoculated Manually. McKINNEY, H. H. Nine lines of winter wheat that have shown either very high resistance or immunity against *Marmor tritici typicum* and *M. t. fulvum* in field tests have been grown in noninfested soil in the greenhouse at temperatures favoring infection. When these lines were inoculated with fresh extracts of the viruses mentioned, strong mosaic symptoms developed in each. The percentage of infected seedlings was sometimes less and sometimes greater than in Michigan Amber, the field-susceptible control variety. Seedlings failing to show mosaic have, on reinoculation, developed mosaic, showing that they escaped infection from the previous inoculation. The results suggest that these wheats possess quantitative resistance to virus, or that they are resistant or immune against some viriliferous vector which inhabits the soil. On the contrary, similar tests with four lines of winter barley that are immune from mosaic in the field indicate that a high percentage of the plants is either highly resistant or completely immune against virus applied by manual inoculation.

Some Little-Known Nematodes Parasitic on Roots. STEINER, G. A cyst-forming *Heterodera* species, which might be mistaken for *H. rostochiensis*, is widely distributed in the potato fields of our eastern and central States. It appears to attack exclusively various polygonums. Normally of lemon shape with a well-marked vulva, certain form variations of the cyst of this knotweed *Heterodera* closely resemble cysts of the golden nematode. The cyst wall, however, shows a linear meandrous ornamentation quite different from that of *H. rostochiensis* with its rows of dots. There is evidence of significant root damage by various species of *Hoplolaimus*. These are large forms causing extensive mechanical injury. Such damage is demonstrated on *Pinus taeda* seedlings. On the roots of a declining gardenia a minimum number of 335 such nematodes was counted per inch. Nematodes that attack roots from the surface have not been given the consideration they deserve. Spiral nematodes (*Helicotylenchus* and *Rotylenchus*), by puncturing the roots, appear regularly to initiate extended necrosis. These forms, dropping from the roots if disturbed, have been largely overlooked. *Rotylenchulus* is a later evolutionary stage of a mode of life initiated by these spiral nematodes: here a permanent fixation on a root surface is attained. Motility is lost and obesity follows sedentary life. Cotton, tobacco, coffee weed, and yew are examples of hosts in the United States.

Combination of Cercospora Leaf-Spot Resistance and Curly-Top Resistance in Sugar-Beet Varieties. STEWART, DEWEY. The leaf-spot-resistant sugar-beet variety U.S. 216, extremely susceptible to curly-top, was crossed with S.L. 611, U.S. 12, and U.S. 22/1, mass selected curly-top-resistant varieties that are extremely susceptible to leaf spot. The F_1's were then crossed with U.S. 22/1, thereby essentially producing a back cross in which the curly-top-resistant variety was the recurring parent. Selections were made from the back-cross generation under conditions of severe leaf-spot exposure, and a further selection for leaf-spot resistance was made in the progeny of this selection. Seed from the second selection for leaf-spot resistance was directly increased at State College, New Mexico, by the field overwintering method. S.P. 3–6–0 tested in 1944 showed curly-top-resistance essentially equal to U.S. 22/1 and only slightly less leaf-spot resistance than U.S. 216. S.P. 456–0, produced in the same manner, when tested in 1946 showed curly-top and leaf-spot resistance essentially equal to that of the respective resistant parents.

Influence of Different Levels and Combinations of Nitrogen, Phosphorus, and Potassium on the Susceptibility of the Tomato Plant to Infection by Alternaria solani. THOMAS, H. REX. Varying the levels and combinations of nitrogen, phosphorus, and potassium in sand-culture greenhouse tests affected the response of tomato plants to infection by *Alternaria solani.* Plants grown on the high level of phosphorus had less defoliation and smaller leaf spots than the plants on the low-phosphorus level. Although plants grown on the high level of nitrogen had larger leaf spots than plants on the low-nitrogen level, there was no significant difference in defoliation between the two levels. Plants grown on the high-potassium level had a greater percentage of dead leaves and larger leaf spots

than plants grown on the low levels in two of the three experiments. A significant interaction between nitrogen and phosphorus was found in the amount of defoliation. With high nitrogen and low phosphorus the plants had the most defoliation and with high nitrogen and high phosphorus, the least. Plants grown in solutions medium to high in nitrogen, low in phosphorus, and medium to high in potassium were usually the most susceptible to disease development. The leaf spots were larger on the older leaves than on younger leaves, regardless of the plants' apparent nutritional condition.

Comparative Action of Viruses and Mutant Chondriogenes on Plant Cells. WOODS, M. W., A. F. WOODS, and H. G. DUBUY. Mutant chondriogenes affect cells in three distinct ways, (1) qualitatively change the structure and function of plastids possessing them, (2) quantitatively change development and function of the non-mutated plastids of the same cell, and (3) alter function and development of the cell as a whole. At least 5 different types of mutant plastids have been observed in a single species. Infection of *Nicotiana tabacum* L. with strains of tobacco-mosaic virus results ultimately in cytological changes paralleling those induced by mutant chondriogenes. In mature cells the virus first affects all of the plastids, reducing their size and pigment content. Physiological disturbances also occur. In time, however, a variable number of plastids become more or less normal in appearance the remainder undergoing profound metamorphosis. Such metamorphosed plastids closely resemble those with mutant chondriogenes. Plastid metamorphosis occurs most rapidly in young systemically infected leaves. In all cases cells occur in which some of the plastids do not undergo such metamorphic change. In chloroses induced by nitrogen starvation or colchicine all of the plastids in a given cell showed general degeneration, and none of the specific changes described for viroses and variegations. Whereas tobacco-mosaic virus is infectious, mutant chondriogenes have not displayed this property.

Following are titles of other research papers presented at the fourth annual meeting of the Potomac Division, the abstracts of which will be published elsewhere:

Preliminary Tests to Determine the Nematocidal and Fungicidal Properties of Certain Chemical Compounds When Used as Soil Fumigants. J. R. CHRISTIE.

Recent Advances in the Breeding of Disease-Resistant Tobaccos. E. E. CLAYTON.

Phytotoxic Effect of DDT and Other Chlorinated Hydrocarbons. ARTHUR C. FOSTER.

Structures Corresponding to Appressoria and Substomatal Vesicles Produced on Nutrient-Solution Agar by Germinating Urediospores of Cereal Rusts. ANNIE MAY KARRER and H. A. RODENHISER.

Inheritance of Resistance to Bacterial Canker in Cow Peas. C. L. LEFEBVRE and HELEN S. SHERWIN.

Dusting of Fruit Trees with Special Reference to Methods of Application. A. A. NIKITIN.

Evidence of Fusion Bodies from Urediospore Germ Tubes of Cereal Rusts. H. A. RODENHISER and ANNIE MAY KARRER.

Physiologic Specialization of Phytophthora Infestans. E. S. SCHULTZ and LILLIAN CASH.

Recent Advances in the Breeding of Disease-Resistant Potatoes. F. J. STEVENSON.

Myrothecium as a Tomato Fruit Rot Organism. J. A. STEVENSON and L. P. McCOLLOCH.

New Methods of Artificial Seed Inoculation for Testing the Resistance of Spring and Winter Barleys Against Stripe, Helminthosporium gramineum. V. F. TAPKE.

A New Local Lesion-Producing Virus of Beans. W. J. ZAUMEYER and H. REX THOMAS.

BOOKS AND WORLD RECOVERY

The desperate and continued need for American publications to serve as tools of physical and intellectual reconstruction abroad has been made vividly apparent by appeals from scholars in many lands. The American Book Center for War Devastated Libraries has agreed to continue meeting this need at least through 1947. The Book Center is therefore making a renewed appeal for American books and periodicals—for *technical and scholarly books and periodicals in all fields* and particularly for *publications of the past ten years*. They will especially welcome complete or incomplete files of PHYTOPATHOLOGY.

The generous support which has been given to the Book Center has made it possible to ship more than 700,000 volumes abroad in the past year. It is hoped to double this amount before the Book Center closes. The books and periodicals which your personal or institutional library can spare are urgently needed and will help in the reconstruction which must preface world understanding and peace.

Ship your contributions to the American Book Center, c/o The Library of Congress, Washington 25, D. C., freight prepaid, or write to the Center for information regarding repayment of shipping charges where prepayment is not possible.
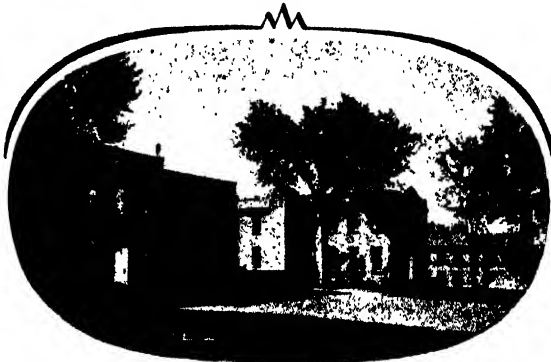
DIAPORTHE PHASEOLORUM VAR. SOJAE ON CROP PLANTS[1]

E . S . L u t t r e l l

Pod blight of lima bean, first reported in 1891, is a widespread and occasionally serious disease along the Atlantic Seaboard. In 1917 Harter (4) demonstrated by means of inoculations that *Diaporthe phaseolorum* (C. & E.) Sacc. is the cause of this disease and also established the connection between the pycnidial and perithecial stages of the fungus. *D. phaseolorum* has subsequently been reported on snap bean (14), pepper (16), and tomato (2, 5).

In 1920 Wolf and Lehman (18) reported a similar pod and stem blight of soybean in North Carolina caused by an undetermined species of *Phoma*. Lehman (8) subsequently produced the perithecial stage of this fungus in culture and named it *Diaporthe sojae* Lehman. He reported that successful inoculations had demonstrated its pathogenicity on soybean. Several years later Wolf and Lehman (19) discovered the perithecial stage of the fungus in the field on over-wintered soybean stems. In a taxonomic study of the genus *Diaporthe*, Wehmeyer (17) reduced *D. sojae* to varietal rank, making it *D. phaseolorum* var. *sojae* (Lehman) Wehmeyer.

The pycnidial stage of *Diaporthe phaseolorum* var. *sojae* has been reported frequently on soybean in various parts of the United States and in the Orient. It has been observed also on cowpea (9, 15), peanut (1), snap bean (9), and lima bean (9). Opinions concerning its importance on soybean have been varied. On the one hand, it has been considered merely a saprophyte (11) or a secondary invader of plants killed by other diseases (6, 12); on the other, it has been reported (3) as causing the death of 20 to 35 per cent of the plants in certain fields. The majority of observers, however, have reported only a trace of damage or losses of up to 0.5 per cent of the crop resulting from infection with this fungus.

In Georgia a fungus determined as *Diaporthe phaseolorum* var. *sojae* was found on soybean, snap bean, cowpea, peanut, lupine, lespedeza, *Strophostyles helvola* (L.) Britton, pepper, tomato, okra, onion, and garlic. The fact that what appeared to be the same fungus was found also on lima bean, the host of *D. phaseolorum*, raised the question of the identity of this fungus as well as the question of whether *D. phaseolorum* and *D. phaseolorum* var. *sojae* may be considered distinct varieties, especially since cross inoculations with the two fungi apparently have never been attempted. A study concerned with the identity and importance of this fungus on the various crop plants on which it has been found was made, therefore, during the period

445
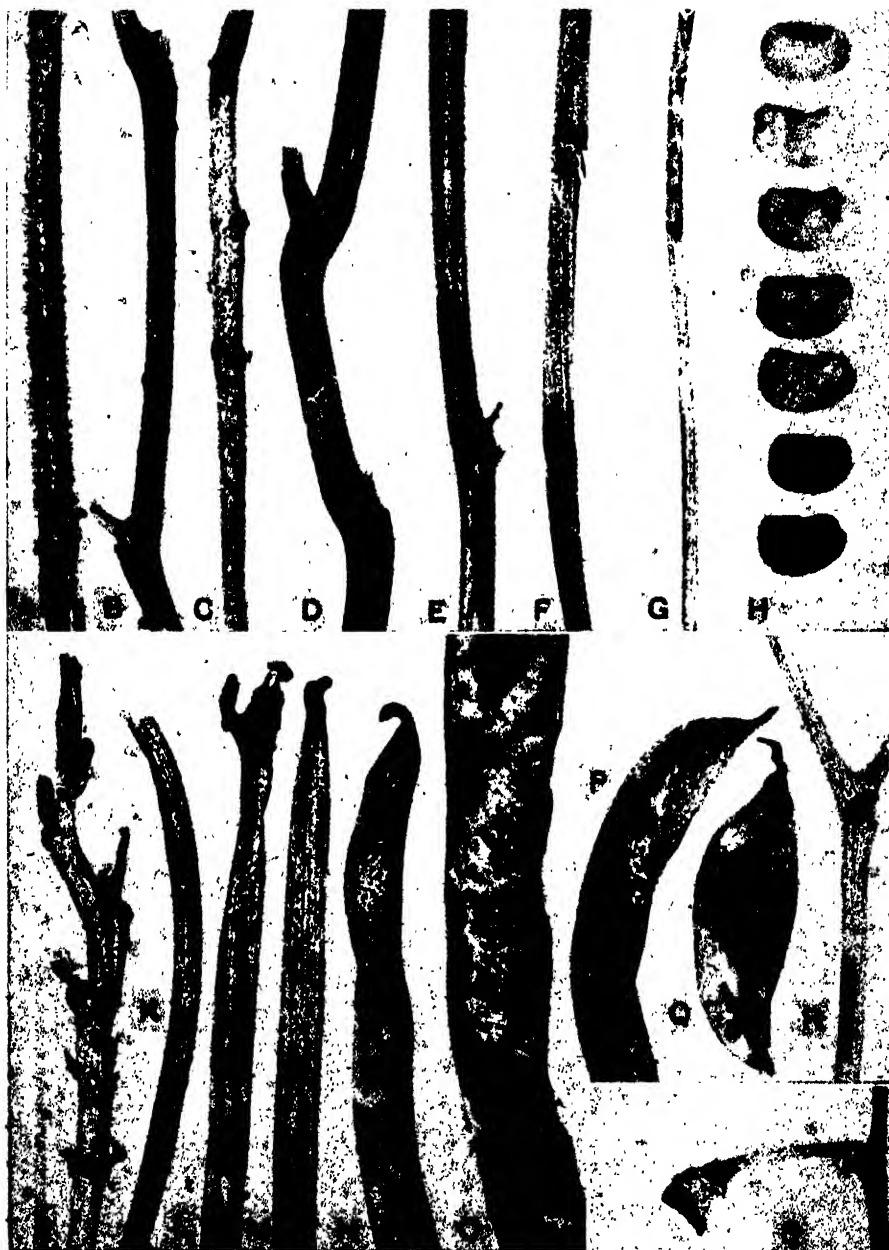
FIG. 1. *Diaporthe phaseolorum* var. *sojae*. A. Perithecia formed on soybean peti-
ole in culture; the perithecial necks developed on the moist lower part of the petiole are
long and coiled, while those toward the drier upper part become progressively shorter.
×1. B. Perithecia developed on over-wintered snap-bean stem; the short perithecial
necks are barely visible. ×1. C. Pycnidia on stem of snap bean inoculated in green-
house. ×1. D. Pycnidia on stem of snap bean from the field. ×1. E. Pycnidia on
soybean stem. ×1. F. Pycnidia on peanut stem. ×1½. G. Pycnidia on lima-bean
stem. ×1½. H. Pycnidia on cowpea seed in various stages of decay. ×1. I. Pycnidia
on stem of cowpea inoculated in greenhouse. ×1. K. Pycnidia on stem of cow
from the field. ×1. L, M. Pycnidia on dead tips of cowpea peduncles. ×1. N,
nidia on cowpea pod. ×1. O, P, Q. Pycnidia on snap-bean pods. ×1. R,
on snap-bean stem. ×1. S. Pycnidia on snap-bean pedicel. ×1½.

from 1943 to 1946 at the Georgia Experiment Station. This study involved a comparison of the morphology, growth in culture, pathogenicity, and host range of *D. phaseolorum* and *D. phaseolorum* var. *sojae*.

HOSTS AND SYMPTOMS

Snap bean. Although *Diaporthe phaseolorum* var. *sojae* occurs chiefly on the stems and pods, it has been found also on the pedicels, leaves, and hypocotyls of snap bean. It first appears on the pedicels of mature plants. Pedicels, from which the pods have been picked or on which the pods have been damaged by insects, die, turn brown, and become covered with small, black, round to elliptical pycnidia which tend to be arranged in rows along the pedicel (Fig. 1, S). Occasionally pedicels bearing immature pods which are dead and shriveled are similarly infected. Rarely the fungus may spread from the pedicels into the peduncle. It does not spread into the vegetative branches, however, until the pods are mature and dried and the plant becomes senescent. As the plant dies the fungus may spread down the stem and fruit on any part. Pycnidia, arranged usually in longitudinal rows, may cover the entire stem (Fig. 1, C, D, R) or occur in localized patches. The fungus may occur on old mature leaves. Then, irregular, brownish, unbordered dead areas covered with pycnidia extend inward from the margins of infected leaflets. *D. phasolorum* var. *sojae*, associated with *Macrophomina phaseoli* (Maubl.) Ashby and *Fusarium spp.*, has been isolated also from the hypocotyls of snap-bean plants dead apparently as a result of root rot. It has been found once fruiting upon a buff-colored, brown-bordered lesion involving the epicotyl and extending down one side of the hypocotyl of a dwarfed, month-old seedling. Generally, however, infection is found only after the pods have dried and the plants begin to die naturally. Pycnidia then appear on the dead stems of plants which have showed no previous infection. Dried pods are similarly invaded. Pycnidia may be more or les scattered over the surface of the pods or may develop only in patches (Fig. 1, O, P, Q). Often they are densely aggregated to form an almost continuous black crust. They are distributed irregularly instead of being arranged linearly as they are on the stems. Seed in infected pods may appear normal or may be shriveled and nonviable. If the pods are moistened by frequent rains or by contact with the ground, the seed may be covered with a white mycelial weft and almost completely decayed.

The fungus occurred indiscriminately on hybrids of a number of snap-bean varieties at both Tifton and Experiment. At both places it was generally associated with *Macrophomina phaseoli*. It was first observed in a field at the Coastal Plain Experiment Station at Tifton on June 21, 1943, on pedicels of plants that otherwise were healthy and on the stems of plants that had matured and died. In the same field in 1944 it was found again on June 1. Observations were made then on five plots of snap bean of the Tendergreen variety. Two pickings of green beans had already been made at the time, but the majority of the plants were still green and bearing

blossoms as well as pods in various stages of maturity. In a sample of 564 of these plants, 78 per cent were green and still bearing. Of these green plants, 42 per cent showed infection of the pedicels with *Diaporthe phaseolorum* var. *sojae*. Of the 22 per cent that were dead, apparently as a result of root rots, pycnidia were found on 10.5 per cent. It was also isolated from hypocotyl tissue of six of a sample of 20 dead plants on which no fruiting bodies of any fungus were evident. Although pedicel infections were not noticed at Experiment, single infected leaves were found here twice during the 1944 growing season. The fungus was commonly present on dead stems and pods at the time of harvesting for seed at both Tifton and Experiment in 1943. It was much less abundant at the same time in 1944, 1945, and 1946.

Cowpea. Diaporthe phaseolorum var. *sojae* has been observed only upon the pods, and, generally to a lesser extent, upon the stems of cowpea. It appears when the first pods become mature and dry. Pycnidia are then found scattered or grouped in patches on the surface of the dried pods (Fig. 1, N). Seed within infected pods may appear normal, or they may be shriveled and killed. Under moist conditions they may be covered with a white mycelial weft. Pods left hanging on the dead plants until late in the season when the pod walls become thin and cracked may contain seed covered with spherical black pycnidia embedded in the mycelial weft over the surface of the seed (Fig. 1, H). From infected pods the fungus spreads into the peduncles as they die. Pycnidia arranged in parallel rows appear on the tips of dying peduncles when the remainder of the plant is green and healthy (Fig. 1, L, M). As the entire plant dies pycnidia develop in patches along the dead stems (Fig. 1, I, K). On cowpea also *D. phaseolorum* var. *sojae* is usually associated with *Macrophomina phaseoli*.

During the fall of 1943 at Experiment only a few scattered infected plants were found. A greater number was found in a large planting of cowpea near Arabi examined in August, 1944, when, although the stems were still green, the majority of the pods were dry and over half of the leaves had been shed. Although stem infections were not common, infection of pods was general. During September and October, 1944, in several plantings of cowpea at Experiment, infection of both stems and pods was general. In one field the infection was greater on cowpea than on soybean and snap bean planted in adjacent rows. Infection was not severe enough to make significant observations on varietal susceptibility. Differences in amount of infection on different varieties seemed to be attributable to the fact that the varieties had matured at different times and, consequently, under different environmental conditions. During September, 1946, pycnidia were found on stems of plants killed by *Sclerotium rolfsii* Sacc. or by *Macrophomina phaseoli* stem canker in a variety planting at Experiment.

Soybean. The fungus is most common on the stems and petioles of soybean, affecting the pods to a lesser extent. Leaf-blade infections are infrequent. The disease first appears on the petioles of lower leaves that are weakened by shading or attacks of other organisms and upon basal

branches that are broken by the weight of pods, wind, or insect damage. Reddish-brown patches of discolored tissue appear on many of these stems, and the stems gradually die. The discoloration appears, however, to be the result of attack by anthracnose. *Colletotrichum glycines* Hori fruits upon the surface of such areas as the stem dies, and pycnidia of *Diaporthe phaseolorum* var. *sojae* then develop upon the intervening areas on the dead stems. *Diaporthe* has not been observed to infect the main stems or upper branches of vigorous plants until after the death of the entire plant following maturity or attack by other organisms such as *Sclerotium rolfsii*. Under favorable conditions dead stems may be covered with pycnidia (Fig. 1, E); while, if conditions are unfavorable, the pycnidia may be limited to small patches, usually formed around the nodes. Pycnidia are often present upon mature dried pods, and the seed in infected pods may be shriveled and dead. Pycnidia occur also upon dried immature pods. Many dried young pods are found, however, whose death appears to be the result of physiological conditions or attack by anthracnose, and it is impossible to be certain that *D. phaseolorum* var. *sojae* is a primary cause of the death of immature pods. On leaves it is sometimes found fruiting upon brownish, unbordered areas of dead tissue extending inward from the tips or margins of the leaflets. *Colletotrichum glycines* is commonly associated with *D. phaseolorum* var. *sojae* on both pods and stems, and no definite lesions which could be ascribed to the latter have been found on soybean.

In the fall of 1943 at Experiment some varieties in a variety planting of soybean were heavily infected, while the majority showed only slight infections. Pycnidia were present on approximately 70 per cent of the dead stems of Boone, the most severely affected variety. Boone was, however, the earliest variety represented in the planting. The pods were mature and dry, and the heavy infection of this variety could be attributed to the fact that it had matured and naturally died earlier than other varieties. During 1944, 1945, and 1946 in the same locality only a few infected pods and stems were found.

Lima bean. *Diaporthe phaseolorum* var. *sojae* has been found only upon dead branches and pods of lima bean (Fig. 1, G). The seed in dried pods may be infected and shriveled when there is no evidence of the fungus on the exterior of the pods. In October, 1943, a few plants of Henderson bush lima bean with infected branches were found at Experiment and on a farm three miles south of Griffin. Pycnidia were present in patches upon the branches, and the fungus was isolated from the tissue of shriveled seed. *Macrophomina phaseoli* also was present on these plants and seemed to be the primary cause of their death. Infection was more abundant on a pole lima bean at Experiment during 1944 and 1945. Pycnidia were present on dead branches of otherwise healthy plants throughout the bearing period. The evidence indicated, however, that *Colletotrichum truncatum* (Schw.) Andrus & Moore was primarily responsible for the death of these branches. Although the dead part of the stem was covered with pycnidia of *D. phaseo-*

lorum var. *sojae*, *C. truncatum* was fruiting at the margin of the lesion toward the base of the branch.

Peanut. *Diaporthe phaseolorum* var. *sojae* occurs upon the stems and stipules of peanut. Pycnidia develop densely in parallel rows over the entire surface of dead stems, giving them a blackened appearance (Fig. 1, F). They may also be formed on dead stipules on yellowed, moribund stems. In August, 1945, the fungus was found on dead stems of Spanish peanuts at two localities. It did not, however, seem to be responsible for the death of these plants. In a planting near Albany it was fruiting on stems killed by a gopher which had eaten the underground parts of the plants. In a planting near Leesburg it was found on the dead stems of plants killed by lightning. In 1946 it was found by Dr. K. H. Garren on dead stems of Spanish peanuts near Arabi and on dead stems of North Carolina Runner peanuts near Metter. *Sclerotium rolfsii* probably caused the death of these plants.

Lespedeza. *Diaporthe phaseolorum* var. *sojae* has been found only on dead stems of lespedeza. The pycnidia were produced sparingly on the slender stems but showed a slight tendency toward an arrangement in longitudinal rows. In October and November, 1945, pycnidia were found on a few plants in a field of Korean lespedeza at Experiment. There was no indication that it was responsible for the death of these plants. Although a majority of the plants were dead or dying, only a few stems bearing pycnidia could be found. Death seemed to be the result of natural senescence.

Strophystyles. In 1945 Dr. C. L. Lefebvre pointed out that pycnidia of *Diaphorthe phaseolorum* var. *sojae* were present on the stems in a collection of dead plants of *Strophostyles helvola* made by H. W. Johnson at Americus, Georgia, April 11, 1939. Examination of a duplicate of this collection in the Georgia Experiment Station herbarium showed that pycnidia of this fungus were scattered on the upper parts of stems of what appeared to be seedling plants. Cause of the death of these plants is unknown.

Lupine. In the spring of 1945 and again in 1946 *Diaporthe phaseolorum* var. *sojae* was found on dead stems of blue lupine at Tifton. The lupine had been planted as a winter crop in a pecan orchard. Pycnidia were observed on stems left standing or on the surface of the ground after the crop had been disced under.

Pepper. *Diaporthe phaseolorum* var. *sojae* has been found on the fruit and the peduncles of pepper. It seems to be capable of acting as a wound parasite, causing a secondary rot of the fruit. The lesions are soft, circular or elliptical, somewhat depressed, and wrinkled. At the advancing margin of the lesion the tissue is blanched and water-soaked. Toward the center it is dry, either blackened or bleached to pale tan, and covered with masses of black pycnidia (Fig. 2, B, D). The pycnidia tend to be arranged in concentric circles, and this arrangement, together with the wrinkling of the host tissue, gives the lesion a zonate appearance. The inner surface of the fruit and the seed are covered with a white mycelial weft. As the rot pro-

FIG. 2, A–G. *Diaporthe phaseolorum* var. *sojae*. A. Pepper fruit decayed by the fungus after invasion through blossom-end rot spot; pycnidia are on the fruit and on the dead peduncle. ×⅔. B. Rot spreading from sunscald spot on pepper fruit; pycnidia have developed in the dead tissue. ×⅔. C. Pycnidia on dried okra pod. ×1. D. Lesion produced on pepper fruit in the field by inoculation through wound now covered with adhesive tape. ×⅔. E. Lesion on picked tomato fruit in moist chamber produced by inoculation through wound. ×⅔. F. Lesion on tomato fruit in the field resulting from natural infection through sunscald spot. ×⅔. G. Cultures producing pycnidia of *D.* (left) and *D. phaseolorum* var. *sojae* (right) on cornmeal mush. ×1.

gresses the fruit shrivels to a dry, wrinkled mass covered with a crust of pycnidia (Fig. 2, A). The fungus may then invade and fruit upon the peduncle (Fig. 2, A). It was fairly common on the fruit of several varieties of bell and hot peppers in plantings at Experiment in 1945 and 1946. The fungus always appeared to have entered the fruit through either blossom-end rot or sunscald spots (Fig. 2, B). Once established in the dead tissue of these spots, however, it spread into the surrounding living tissue and rotted the fruit. This fruit rot has been duplicated by artificial inoculations in wounds (Fig. 2, D).

Tomato. Acting as a wound parasite, *Diaporthe phaseolorum* var. *sojae* is able to cause a rot of tomato fruit. The lesions are circular, slightly depressed, water-soaked areas (Fig. 2, E, F). The infected tissue is discolored. On green fruits it is dark green; on ripe fruits it is dark red. Ultimately the fruit is reduced to a soft-rotted, wrinkled, blackened mass with pycnidia scattered over its surface. Natural infection of tomato in the field was observed once in 1946 on a few fruits in a home garden at Experiment (Fig. 2, F). The fungus had entered through sunscald spots. A similar rot has easily been produced, however, by inoculating the fungus into wounds on green or ripe fruits (Fig. 2, E).

Okra. In December, 1945, a single pod of okra infected with *Diaporthe phaseolorum* var. *sojae* was found near Experiment. The pycnidia of the fungus were aggregated to form a small black patch on the surface of the dead pod (Fig. 2, C). This pod had been left hanging on the plant in the field after maturity. The fungus appeared merely to have invaded the pod after it had matured and died naturally.

Onion and Garlic. Pycnidia of *Diaporthe phaseolorum* var. *sojae* were found on dead tips of leaves of onion and garlic plants in home gardens at Experiment in the spring of 1946. Only a few leaves were infected.

ETIOLOGY

Morphology. The pycnidium of *Diaporthe phaseolorum* var. *sojae* originates beneath the host epidermis as a stromatic mass of pseudoparenchymatous cells. Across the middle of this stroma a layer of fertile cells develops. These cells become elongated to form short, simple, unicellular conidiophores. As a result of their growth the stroma is split to produce a slit-like cavity into which the conidiophores project from all sides. Pycnospores are abstricted singly from the tips of the conidiophores and fill the cavity. As the pycnospores develop, the cells which produced them disintegrate, and cells of the stroma beneath them in turn become fertile. The cavity is thus gradually enlarged until only the outer layers of the stroma remain to form the wall of the pycnidium. The wall, therefore, varies in thickness, becoming gradually thinner as the pycnidium matures. At maturity the wall is composed of a layer of three to six brown, thick-walled, flattened cells surrounding the central cavity. Toward the apex the wall is thicker and composed of isodiametric cells, forming a short, rounded or conical, ostiolate beak. The

cavity is lined with a layer two to four cells thick of hyaline, isodiametric cells from which the condiophores arise. Pycnidia usually contain only a single cavity. Pycnidia with two or three cavities occur commonly, however, and pycnidia containing five to ten cavities may be found occasionally. Often the walls between two cavities are disintegrated, forming a single cavity which is, however, provided with two separate ostioles.

In surface view pycnidia on stems are elliptical with the longer axis parallel to the axis of the stem. On leaves and pods they are round to oval. In sections the pycnidia on stems and pods are lenticular, while on leaves they are more nearly spherical. Pycnidia varied greatly in size on the same host, being much smaller on leaves than on stems and pods. There seemed, however, to be no difference in size of pycnidia correlated with difference in host. For example, on snap-bean leaves pycnidia measured 95–245 × 82–163 μ (mean 148 × 122 μ), on snap-bean pods 163–408 × 136–340 μ (mean 283 × 236 μ), and on soybean pods 190–490 × 177–313 μ (mean 275 × 246 μ).

Pycnospores of *Diaporthe phaseolorum* var. *sojae* are 1-celled, hyaline, elliptical, and rounded at both ends. They contain a single central nucleus and usually a single conspicuous oil globule in either end of the spore. Occasionally three to four oil globules are present. There was little difference in size of pycnospores from different parts of the same host or from different hosts.

The stylospores differ from pycnospores in that they are long, filiform, usually curved spores lacking the conspicuous oil globules which are characteristic of the pycnospores. Spores intermediate between typical stylospores and typical pycnospores occur frequently. Stylospores have been found only a few times and then usually in late fall. They have been found only on soybean, cowpea, and lima bean. They occur so rarely, however, that this is considered to be merely a matter of chance.

Pycnidia of *Diaporthe phaseolorum* are similar in structure and dimensions to those of *D. phaseolorum* var. *sojae* (Table 1). On lima-bean stems, pycnidia of *D. phaseolorum* measured 136–490 × 122–326 μ (mean 242 × 201 μ), those of the variety *sojae* 136–408 × 109–299 μ (mean 245 × 177 μ). Pycnospores of *D. phaseolorum* are longer and wider both in mean and maximum dimensions than those of the variety *sojae* (Table 1). The difference in size is slight, however, and sometimes samples of pycnospores of *D. phaseolorum* approach samples of pycnospores of the variety closely in size. For example, the lowest measurements recorded for samples of *D. phaseolorum* pycnospores were 6.0–11.3 × 2.7–3.5 μ (mean 8.0 × 3.0 μ); the highest measurements for samples of pycnospores of the variety *sojae* were 6.0–9.5 × 2.3–3.3 μ (mean 7.8 × 3.0 μ). Stylospores of *D. phaseolorum* are distinctly larger than those of the variety *sojae* (Table 1).

. Perithecia of *Diaporthe phaseolorum* var. *sojae* were found in the field on over-wintered stems of soybean, cowpea, lima bean, and snap bean (Fig. 1, B). Perithecia developed also within two months on infected stems of soybean and cowpea brought into the laboratory in August and in October and placed in beakers partly filled with water.

TABLE 1.—A comparison of the morphology of Diaporthe phaseolorum and D. phaseolorum var. sojae

Structure	On host		In culture		
	D. phaseolorum[a]	D. phaseolorum var. sojae	D. phaseolorum	D. phaseolorum var. sojae (perithecial strains)	D. phaseolorum var. sojae (nonperithecial strains)
			Separate	In stroma	In stroma
Pycnidia	136–639 × 122–354μ	95–598 × 82–408μ			
Grand mean	(50) 267 × 230μ	(400) 240 × 185μ			
Variation in sub-means[b]	242–332 × 201–259μ	148–297 × 122–246μ			
Pycnospores	5.3–13.0 × 2.5–4.5μ	4.5–9.8 × 1.1–3.9μ	5.3–13.5 × 2.3–6.0μ	4.5–9.0 × 1.8–3.6μ	4.5–9.0 × 1.6–3.3μ
Grand mean	(175) 8.6 × 3.1μ	(650) 7.3 × 2.7μ	(100) 7.9 × 3.1μ	(525) 6.6 × 2.7μ	(600) 6.4 × 2.3μ
Variation in sub-means	7.9–9.2 × 2.7–3.6μ	6.4–8.0 × 1.8–3.0μ	7.4–9.2 × 3.0–3.4μ	6.2–7.4 × 2.4–2.9μ	5.8–7.2 × 1.9–2.7μ
Stylospores	14.0–30.8 × 1.4μ	9.0–21.0 × 0.8–1.8μ	15.0–61.5 × 1.2–2.3μ	9.8–22.6 × 0.7–1.6μ	None
Grand mean	(25) 21.4 × 1.4μ	(59) 14.3 × 1.3μ	(100) 34.1 × 1.5μ	(525) 15.8 × 1.3μ	
Variation in sub-means		12.9–15.3 × 1.3–1.4μ	27.0–39.8 × 1.4–1.5μ	12.9–19.7 × 1.1–1.4μ	
Perithecia	158–356μ	163–272 × 190–340μ	None	In stroma	None
Grand mean	252μ	(100) 218 × 254μ	do		do
Variation in sub-means		199–255 × 243–291μ	do		do
Asci	28.0–46.2 × 5.2–8.0μ	28.0–44.8 × 7.8–10.6μ	do		do
Grand mean	37.4 × 6.7μ	(75) 38.4 × 8.6μ	do		do
Variation in sub-means		38.3–38.5 × 8.6μ	do		do
Ascospores	6.4–12.0 × 2.3–4.0μ	9.0–13.5 × 3.0–4.8μ	do	8.0–13.5 × 2.9–5.3μ	do
Grand mean	9.5 × 2.9μ	(100) 11.1 × 3.7μ	do	(525) 10.2 × 3.4μ	do
Variation in sub-means		10.8–11.6 × 3.5–4.0μ	do	9.6–12.0 × 3.1–4.2μ	do

[a] Measurements of perithecia, asci, and ascospores of D. phaseolorum are from Harter (4).
[b] Sub-means are means of samples of 25 measurements each. The number of measurements from which each grand mean is derived is indicated in parentheses preceding the mean.

The perithecium originates from a coiled, multicellular ascogonium which develops from the mycelium in the inner cortex of the host. The ascogonium becomes enveloped by a spherical mass of hyphae which form the wall of the perithecium. The interior of the perithecium is filled with a mass of large, hyaline, pseudoparenchymatous cells. The ascogenous hyphae are embedded in the center of this pseudoparenchyma, and from them the asci arise. As the asci expand, the pseudoparenchyma is crushed and disintegrated, and the asci push down into the base of the perithecium. At the time the asci begin to develop, hyphae in the apex of the perithecial wall grow upward in a column, forming the ostiolate neck of the perithecium. The neck extends tortuously through the cortex and epidermis to the exterior of the host stem (Fig. 1, A, B). Development of the perithecium appears to be essentially similar to that of *Ceratostomella adiposum* (Butl.) Sartoris (13). The perithecia are embedded singly in the host tissues. The limits of growth of the mycelium are marked by a black line in the host tissue.

Mature perithecia are roughly spherical but are usually flattened at the base so that they are more wide than high. The walls surround a central cavity filled across the lower two-thirds with a mass of aparaphysate asci. The asci are clavate, and each contains eight ascospores. They break loose at the base and their walls disintegrate, liberating the spores which, embedded in a viscous fluid, ooze out through the ostiole. The ascospores are hyaline, uniseptate, elliptical, rounded at either end, and slightly constricted at the septum. Each cell of the ascospore is uninucleate and contains one large and one small oil globule. The length of the perithecial neck varies, depending upon the moisture conditions under which it develops. This was demonstrated in culture on soybean petioles in culture tubes partially filled with water. Toward the moist base of the petiole the necks were long and coiled, toward the drier upper portion they became progressively shorter (Fig. 1, A). There was little difference in the perithecial stage of the fungus on the four different hosts.

No perithecia of *Diaporthe phaseolorum* have been examined. From Harter's (4) description, however, it is apparent that they are essentially similar to perithecia of the variety *sojae*. They differ slightly in width of asci and in size of ascospores, those of *D. phaseolorum* being the smaller (Table 1).

Growth in culture. Diaporthe phaseolorum var. *sojae* was isolated from all recorded hosts, with the exception of *Strophostyles helvola*. Of these isolates, 45 have been studied in culture. They were obtained from the vicinity of Experiment in middle Georgia and from the vicinities of Arabi, Tifton, Albany, and Leesburg in south Georgia. Dr. L. L. Harter kindly furnished an isolate of *D. phaseolorum* obtained from lima bean in Maryland. Material of this fungus was not found in Georgia until 1946. The cultures were maintained on three per cent malt agar. The fungi fruited more abundantly, however, on snap-bean stems and soybean petioles partially immersed in water in culture tubes and on cornmeal mush. Perithecia

were produced more readily in cool weather. In making comparisons of pycnidia and perithecia in culture, the isolates were grown at room temperature on cornmeal mush in 250-cc. flasks.

Isolates of *Diaporthe phaseolorum* var. *sojae* formed a white, flocculent, rapidly growing mycelium. There was a tendency for the aerial hyphae to become loosely united in slender strands. The mycelium of *D. phaseolorum* was similar but was more closely appressed, and the hyphae did not form strands. It also grew more slowly. The most striking differences between the two fungi appeared, however, in the production of fructifications. As previously described by Harter (4), colonies of *D. phaseolorum* produced only pycnidia in culture. Perithecia were never formed on any medium employed. The pycnidia were scattered singly over the surface of the medium enmeshed in the mycelial weft. They were composed of a thin wall of loosely compacted hyphae surrounding a large central cavity in which pycnospores and later stylospores were produced abundantly. The spores oozed from the pycnidia and collected in large yellowish or orange droplets at the mouths of the ostioles (Fig. 2, G, left).

On cornmeal mush and on agar media pycnidia of *Diaporthe phaseolorum* var. *sojae* were produced in groups in conspicuous, black, pulvinate stromata formed on the mycelium (Fig. 2, G, right). The stromata were 1–9 mm. in diameter and often were produced so abundantly that they almost covered the white mycelium with a black crust. The interior of the stroma was plectenchymatous, the peripheral portions were pseudoparenchymatous. Pseudoparenchymatous pycnidia were embedded in the surface of the stroma with their beaks more or less protruding. Pycnospores were produced abundantly in most cultures, but they rarely oozed from the ostioles in conspicuous globules as in *D. phaseolorum*. Although pycnospores of both fungi in culture were smaller than those produced in the field, the same relative difference between them was maintained. The difference in size of stylospores was greater in culture than in the field (Table 1). In some strains perithecia were produced later in the same stromata beneath the pycnidia. It is interesting to note that, while on the host stems in nature the perithecia are embedded separately in the host tissue, in culture they are grouped in a diatrypoid stroma.

Although all isolates of *Diaporthe phaseolorum* var. *sojae* were alike in the production of stromata and thus distinct from isolates of *D. phaseolorum*, they varied widely among themselves. The various isolates were often recognizable individuals. Size of stromata varied greatly. Some isolates formed almost smooth or slightly botryose stromata; in others the stroma became echinulate through the production of long pycnidial beaks. Some were almost sterile; others produced pycnospores abundantly. As Lehman (8) found previously, some isolates produced only pycnospores; others produced stylospores as well. Perithecia were developed by only about half of the isolates. It was soon apparent that there was a correlation among some of these characters. All isolates could be divided into two

major groups, the perithecial strains and the nonperithecial strains (Table 1). The perithecial strains always produced perithecia on all media, formed stylospores, and showed a tendency to form the echinulate type of stroma. The nonperithecial strains never produced perithecia under any conditions, never formed stylospores on the usual media, and tended to form smooth or botryose stromata. Many of them produced pycnospores only sparingly. Of the 45 isolates tested, 21 were perithecial strains and 24 were nonperithecial strains. Perithecial strains predominated among isolates from middle Georgia. Only 12 of the 31 isolates from this area were nonperithecial, while 12 of the 14 isolates from south Georgia were nonperithecial strains.

The failure of many of these isolates to form perithecia probably cannot be attributed to the existence of different sexual strains, because Tucker (16) has demonstrated that *Diaporthe phaseolorum* var. *sojae* (referred merely to the *D. phaseolorum* complex) is homothallic. The production of both perithecial and nonperithecial strains seems to be common among species of *Diaporthe*. It has also been reported by Leach (7) in *Ceratostomella ips* Rumb. Leach was unable to induce the formation of perithecia in *C. ips* by mating nonperithecial strains. In *D. phaseolorum* var. *sojae* mating of two nonperithecial strains from Experiment and two from Tifton likewise failed to induce formation of perithicia. Nonperithecial strains possibly are not incapable of producing perithecia. They merely have not done so under cultural conditions.

By using the method of Nitimargi (10) it has been possible to demonstrate that at least some of the nonperithecial strains are capable of producing stylospores although they do not produce them on ordinary media. Two lots of medium were prepared. Medium A contained 2 gm. sucrose, 2 gm. asparagin, 0.75 gm. magnesium sulfate, 1.25 gm. potassium phosphate, 15 gm. agar, and 1,000 cc. water. Medium B was the same except that the amount of sucrose was increased to 128 gm. Eight tubes of each medium were inoculated with each of three perithecial and five nonperithecial strains. Half of the resulting cultures were kept in total darkness, the other half were exposed to normal daylight. Since all isolates fruited poorly on both media, the experiment was continued for three months. Of the cultures kept in total darkness none of the isolates on medium B fruited and only one of medium A formed pycnospores. This was a nonperithecial strain, and it did not form stylospores. The results from cultures exposed to daylight were as follows: on medium A one of the three perithecial strains produced pycnospores and stylospores, and three of the five nonperithecial strains produced pycnospores but no stylospores. On medium B one of the perithecial strains and two of the nonperithecial strains produced both pycnospores and stylospores. One of the nonperithecial strains produced only a few stylospores, but the other produced them in the ratio of one stylospore to four pycnospores. Measurements of spores from this culture were as follows: pycnospores, 5.3–7.7 × 2.0–2.5 μ (mean 6.6 × 2.3 μ); stylo-

spores, 9.3–26.6×0.8–1.3 μ (mean 21.4×1.1 μ). These measurements are close to those of spores produced normally by perithecial strains (Table 1).

Contrary to results obtained by Lehman (8), exposure to light was found unnecessary for the formation of pycnidia, although it did stimulate their production. Fresh transfers of the isolates to be tested were placed in total darkness for five days. Flasks of cornmeal mush and tubes of soybean petioles were inoculated from these cultures. They were then divided into two lots. One lot was exposed to light from a north window; the other was kept in total darkness for one month. The isolate of *Diaporthe phaseolorum* formed pycnospores and stylospores in darkness as well as when exposed to light. Of the 17 isolates of *D. phaseolorum* var. *sojae* tested, only four failed to form pycnospores in darkness, and these were nonperithecial strains which formed pycnospores poorly when exposed to light. The perithecial strains produced stylospores and perithecia also in total darkness. All isolates, however, fruited more abundantly when exposed to light.

Inoculations. Inoculations with pycnospores from cultures of various isolates of *Diaporthe phaseolorum* and *D. phaseolorum* var. *sojae* were made on snap bean, cowpea, lima bean, soybean, peanut, tomato, and pepper. The plants were grown in sterile soil in 8-inch pots with one to four plants in each pot. Approximately half of the plants were wounded on stems and pods with a razor blade. Pycnospore suspensions were then applied to all parts of the plants. After the inoculum had dried, the plants were placed in a moist chamber for 48 hours. They were then returned to benches in the greenhouse and kept under observation for at least two months. Controls run in all experiments were treated similarly except for the fact that they were not inoculated. Pepper and tomato fruits were inoculated in the field by inserting mycelium in wounds on green and ripe fruits that had been surface sterilized with 95 per cent alcohol. The wounds were then covered with adhesive tape. Inoculations were made on picked fruit by inserting mycelium in wounds on fruit that had been surface sterilized with mercuric chloride solution and placed in large, sterile culture dishes. Sterile agar was placed in wounds in control fruit. The results of these inoculations are presented in table 2.

Diaporthe phaseolorum infected only lima bean. Characteristic lesions, as described by Harter (4), appeared on the immature pods at the end of one week and were well developed, killing the pods, two weeks after the inoculations were made. Leaf infections were not obtained. The fungus entered the peduncles from infected pods and spread down the branches of the plants only after they became senescent. A higher percentage of infection would have been obtained except for the fact that on some plants the pods were so nearly mature that they began to dry before lesions developed on them. Inoculations on half-grown pods were always successful. Infection took place through the nonwounded epidermis more readily than through wounds. *D. phaseolorum* did not produce lesions on any other plants and never fruited on them after they had died from other causes.

It did not infect either pepper or tomato fruits even when inoculated into wounds.

Diaporthe phaseolorum var. *sojae* never infected seedling plants and never produced lesions on mature plants of any host with the exception of pepper and tomato. At the end of two months it was fruiting on many dead stems of all hosts (Fig. 1, C, I). By this time many of the plants were senescent or had died naturally, as might be expected since they were fully mature when they were inoculated. It is significant that the number of dead plants usually exceeded the number of infected plants and that often a greater number of control plants than of inoculated plants was dead. Infection with *Macrophomina phaseoli*, resulting presumably from wind-borne pycnospores, caused the death of some plants. After the control plants and some of the plants inoculated with *D. phaseolorum* died, *D. phaseolorum* var. *sojae* fruited on them. Apparently it spread readily from inoculated plants that had died earlier. Infection of wounded green or ripe pepper (Fig. 2, D) and tomato (Fig. 2, E) fruits with *D. phaseolorum* var. *sojae* was consistently obtained. Infection produced a fruit rot similar to the rot of these fruits resulting from natural infection in the field. The fungus did not, however, invade ripe, nonwounded tomato fruits when they were placed in contact with rotted fruits in a culture dish.

Because Lehman (8) had reported successful inoculations on soybean with *Diaporthe phaseolorum* var. *sojae*, some additional inoculations were made on this host. The host material was large, fully mature soybean plants grown singly in 8-inch pots. These were divided into two lots of which one was inoculated with a suspension of pycnospores from soybean stems naturally infected in the field, while the other lot was retained as controls. Half of the plants in each lot were wounded on stems and pods. The plants were placed in a moist chamber for 48 hours and then removed to a cloth house where they were exposed to a fine spray of water every night for two weeks and were kept moist for two weeks thereafter. Results at the end of one month were as follows: of six wounded, inoculated plants six were infected; of six nonwounded, inoculated plants two were infected; of six wounded, control plants none was infected; and of six nonwounded, control plants none was infected. At this time all plants, both inoculated and control, were dead. *Glomerella glycines* (Hori) Lehman and Wolf, accompanied by *Fusarium sp.* and various molds, was fruiting on all of these plants. Under these conditions of prolonged high humidity, some infection with *D. phaseolorum* var. *sojae* was obtained; but at the same time various saprophytic fungi also fruited on the same plants. Since the control plants died as soon as the inoculated plants, it is impossible to conclude that *D. phaseolorum* var. *sojae* was responsible for the death of the plants on which it fruited.

Since *Diaporthe phaseolorum* var. *sojae* was isolated from snap-bean plants killed by root rot, inoculations were made to determine whether it might infect seedlings through the soil. In the first test, pots of sterile soil

TABLE 2.—*Results of inoculations with Diaporthe phaseolorum and D. phaseolorum var. sojae on various crop plants*

Host	Plants inoculated	Results			
		One month		Two months	
		Plants dead	Plants infected	Plants dead	Plants infected
	number	*per cent*	*per cent*	*per cent*	*per cent*
Inoculated with *D. phaseolorum* var. *sojae*					
Snap bean					
Seedling	4	0	0	0	0
Mature	76	3.9	1.3	15.8[a]	13.2
Cowpea					
Seedling	8	0	0	0	0
Mature	53	3.8	1.9	71.7[b]	34.0
Lima bean					
Seedling	1	0	0	0	0
Mature	54	0	1.9	16.7[c]	7.4
Soybean					
Seedling	21	0	0	0	0
Mature	33	0	0	57.6	57.6
Peanut					
Mature	4	0	0	0	0
Tomato					
Seedling	8	0	0	0	0
Fruit	20		25.0	..	..
Picked fruit					
Green	22		63.9	..	..
Ripe	7		100.0	..	..
Pepper					
Seedling	10	0	0	0	0
Mature	10	0	0	0	0
Fruit	21		81.0	...	..
Picked fruit	22		0	.	.
Inoculated with *D. phaseolorum*					
Snap bean					
Seedling	4	0	0	0	0
Mature	19	10.5	0	21.0	0[d]
Cowpea					
Seedling	8	0	0	0	0
Mature	28	0	0	7.1	0[e]
Lima bean					
Seedling	6	0	0	0	0
Mature	31	0	54.8	0	54.8
Soybean					
Seedling	14	0	0	0	0
Mature	16	0	0	50.0	0[f]
Peanut					
Mature	2	0	0	0	0
Tomato					
Seedling	4	0	0	0	0
Fruit	10	....	0	.....	....
Picked fruit					
Green	12	....	0	....	....
Ripe	3	....	0	....	....
Pepper					
Seedling	5	0	0	0	0
Mature	5	0	0	0	0
Fruit	10	....	0	....	....
Picked fruit	11	....	0	....	....

TABLE 2. (*Continued*)

Host	Plants inoculated	One month		Two months	
		Plants dead	Plants infected	Plants dead	Plants infected
	number	*per cent*	*per cent*	*per cent*	*per cent*
Noninoculated control					
Snap bean					
Seedling	4	0	0	0	0
Mature	19	0	0	42.1	42.1f
Cowpea					
Seedling	4	0	0	0	0
Mature	19	21.0	0	57.9	15.8g
Lima bean					
Seedling	2	0	0	0	0
Mature	15	0	0	0	0
Soybean					
Seedling	7	0	0	0	0
Mature	14	0	0	78.6	21.4g
Peanut					
Mature	2	0	0	0	0
Tomato					
Seedling	4	0	0	0	0
Fruit	10		0		
Picked fruit					
Green	7		0		
Ripe	3		0		
Pepper					
Seedling	5	0	0	0	0
Mature	5	0	0	0	0
Fruit	12		16.7g		
Picked fruit	11		0		

ᵃ Four plants killed by *Macrophomina phaseoli*.
ᵇ Ten plants killed by *M. phaseoli*.
ᶜ All killed by *M. phaseoli*.
ᵈ Two plants infected with *D. phaseolorum* var. *sojae*.
ᵉ One plant infected with *D. phaseolorum* var. *sojae*.
ᶠ Eight plants infected with *D. phaseolorum* var. *sojae*.
ᵍ All infected with *D. phaseolorum* var. *sojae*.

were inoculated with a culture of the fungus grown on sterile oats. Sterile oats were mixed with the soil in control pots. Of 40 snap-bean seeds planted in inoculated soil, 37 emerged. At the end of two months 33 were healthy mature plants. Four plants were killed by a *Pythium sp*. Of 20 seeds planted in control pots, 17 emerged and 16 plants survived to maturity. One was killed by *Pythium sp*. In the second test, seedling snap-bean plants growing in sterile soil in 8-inch pots were inoculated by digging into the soil around the plants and placing oats, on which the fungus was growing, in contact with the roots and hypocotyls. Sterile oats were placed around the control plants. Of 28 inoculated plants, 22 were healthy two months later. Six had been killed by *Pythium sp*. Of 13 control plants, 9 survived to maturity, 4 being killed by *Pythium sp*. *D. phaseolorum* var. *sojae* never fruited on any of these plants.

DISCUSSION

Host range. *Diaporthe phaseolorum* is limited to lima bean, the host on which it was orginally described. Inoculation experiments have not confirmed reports of its occurrence on any other host. Tucker (16) referred the fungus causing a fruit rot of pepper in Texas merely to the group that Wehmeyer (17) included in *D. phaseolorum.* Because of the dimensions of pycnospores and stylospores and the production of stromata and perithecia in culture, it is evident that this fungus was *D. phaseolorum* var. *sojae.* It likewise seems certain that the fungus Bratley and Wiant (2) found on tomatoes in New York markets was this variety. Hopkins (5) stated only that the fungus he found on tomato in Rhodesia resembled *D. phaseolorum.* It is possible that this also was the variety *sojae*, but too little information was furnished to determine its identity. Seymour's (14) report of *D. phaseolorum* on snap bean apparently was a mistake. No other reference to its occurrence on this host has been found in the literature. A specimen collected by W. D. Moore at Cairo, Georgia, June 20, 1931, and labelled *D. phaseolorum* on *Phaseolus vulgaris* was obtained from the Bureau of Plant Industry Mycological Collections. This proved to be *Macrophomina phaseoli* on lima-bean stems.

On the other hand, *Diaporthe phaseolorum* var. *sojae* has been found on 13 different hosts, and it is probable that this list of hosts will be extended. Although there were variations among specimens of the fungus on different hosts and on different host parts and among various isolates of the fungus in culture, they all seem referable to *D. phaseolorum* var. *sojae.*

Pathogenicity and importance. My inoculations show, as did those of Harter (4), that *Diaporthe phaseolorum* is pathogenic to lima bean. It may enter non-wounded immature pods and produce characteristic lesions on them within two weeks. The inoculations have not shown that *D. phaseolorum* var. *sojae* is a primary pathogen on any of its hosts. It may cause a rot when inoculated into wounds in pepper and tomato fruits. Under natural conditions, however, it always appears to be secondary, entering the fruits through blossom-end rot or sunscald spots. On all other hosts this fungus seems to be merely a vigorous saprophyte, quickly fruiting on plants or plant parts killed or greatly weakened by other organisms or by adverse growing conditions. It may then appear to have caused the death of the plants on which it is fruiting, but careful study will usually reveal that other environmental factors are primarily responsible. There was no evidence that soybean is more susceptible to infection with *D. phaseolorum* var. *sojae* than any other hosts on which it occurs, such as snap bean and cowpea. Lehman (8) obtained infection of soybean for the most part by keeping inoculated plants covered with bell jars for long periods. Furthermore, his results were not checked against control plants. Under similar conditions of high humidity for a long period, *D. phaseolorum* var. *sojae* has been induced to fruit on pods and stems of dying soybean plants. This can hardly be considered proof of the pathogenicity of *D. phaseolorum* var. *sojae*, however, because under these conditions various saprophytic fungi

also fruited on both inoculated and control plants. This fungus must, therefore, have little influence upon crop yields. Under favorable conditions it might cause some crop loss through decay of seed, but such losses have not yet been found in Georgia.

Taxonomy. Because of the differences in pathogenicity and in host range between *Diaporthe phaseolorum* and *D. phaseolorum* var. *sojae*, it is desirable that these two fungi be recognized taxonomically. The question remaining is what taxonomic category should be employed, and this must be answered by a consideration of comparative morphology. On the host no differences in structure or dimensions of pycnidia or perithecia of the two fungi have been found. Stylospores of *D. phaseolorum* were much larger than those of the variety *sojae*, but they were found so rarely in field material of either fungus that this distinction is of little practical importance. Pycnospores of *D. phaseolorum* were slightly larger, exceeding those of the variety by 2.2 μ in maximum length and by 1.3 μ in mean length. The differences in maximum and in mean widths were 0.6 μ and 0.4 μ, respectively (Table 1). On the other hand, the asci of the variety were slightly wider and the ascospores slightly larger than those of the species, the difference in ascospore dimensions being in both maximum and mean length 1.5 μ and in both maximum and mean width 0.8 μ. In culture the two fungi are readily distinguished because of the production of pycnidia in pulvinate stromata in cultures of *D. phaseolorum* var. *sojae*. The difference in size of stylospores in culture is obvious (Table 1), but more than half of the isolates of the variety did not produce stylospores at all. *D. phaseolorum* did not form perithecia in culture, but neither did many isolates of *D. phaseolorum* var. *sojae*. Although the pycnospores produced in culture by both fungi were smaller than those produced in nature, the same relative difference was maintained (Table 1). Nevertheless, the difference was slight, and means of small samples of pycnospores of the two fungi occasionally overlapped. Also differences in size of pycnospores of each fungus in field material and in culture were almost as great as the differences between the two fungi under the same conditions.

Although these differences in spore size are generally sufficient to distinguish the two fungi, positive determination of *Diaporthe phaseolorum* and *Diaporthe phaseolorum* var. *sojae* upon the basis of spore measurements might not always be possible. Positive separation of them depends primarily upon differences in pathogenicity and in appearance in culture. Since these differences are accompanied by slight differences in morphology, *D. phaseolorum* var. *sojae* should be considered a well established variety. These differences might be considered sufficient to warrant specific rank. It seems best, however, to follow Wehmeyer's (17) treatment of the genus *Diaporthe*, maintaining this fungus as a variety of *D. phaseolorum*.

SUMMARY

Although *Diaporthe phaseolorum* (C. & E.) Sacc. has been reported on tomato, pepper, and snap bean, inoculations have shown that it is limited

to lima bean, the host on which it was originally described. On lima bean it is definitely pathogenic, entering through the non-wounded epidermis of immature pods and producing characteristic lesions.

Diaporthe phaseolorum var. *sojae* (Lehman) Wehmeyer has been found on the following hosts: soybean, snap bean, cowpea, lima bean, peanut, lupine, lespedeza, *Strophostyles helvola*, tomato, pepper, okra, onion, and garlic. Acting as a wound parasite, it is capable of causing a rot of pepper and tomato fruits. It enters these fruits through blossom-end rot or sun-scald spots. There is no evidence from observations or inoculations that it is pathogenic to any other host. It appears to be merely a vigorous sapro-phyte that may quickly fruit upon plants killed by other organisms or by adverse growing conditions. It might cause crop losses through the decay of seed of plants such as soybean, cowpea, snap bean, and lima bean; but it has not been observed to do so in Georgia.

Diaporthe phaseolorum var. *sojae* differs from *D. phaseolorum* primarily in pathogenicity and in the production in culture of pycnidia, and in some strains perithecia, in conspicuous diatrypoid stromata. Stylospores of the var. *sojae* are smaller than those of *D. phaseolorum*, but these spores are rarely produced in nature by either fungus. The ascospores of the variety are slightly larger and the pycnospores slightly smaller than those of the species. Because the morphological differences are slight, the classification of this fungus as a variety of *D. phaseolorum* has been accepted.

In culture, isolates of *Diaporthe phaseolorum* var. *sojae* may be divided into perithecial and nonperithecial strains. Perithecial strains produce pycnospores, stylospores, and perithecia and tend to form echinulate stro-mata. Nonperithecial strains never produce either stylospores or perithecia and tend to form smooth or botryose stromata. They often produce pycno-spores only sparingly. Within each strain individual isolates vary in size and shape of stromata, in fertility, and in general appearance. Of 45 isolates obtained from various hosts in Georgia, 21 were perithecial strains, 24 were nonperithecial strains.

BOTANY DEPARTMENT,
  GEORGIA EXPERIMENT STATION,
    EXPERIMENT, GEORGIA.

LITERATURE CITED

1. ATKINSON, R. E. Report on diseases of peanut. U. S. Dept. Agr., Pl. Dis. Reptr. **28**: 1096. 1944.
2. BRATLEY, C. O., and JAMES S. WIANT. Diseases of fruits and vegetables on the New York Market during the months of April to September, 1940, inclusive. U. S. Dept. Agr., Pl. Dis. Reptr. **25**: 68–71. 1941.
3. BRETZ, T. W. Surveys in Iowa and Missouri. U. S. Dept. Agr., Pl. Dis. Reptr. **27**: 379–380. 1943.
4. HARTER, L. L. Podblight of the lima bean caused by *Diaporthe phaseolorum.* Jour. Agr. Res. [U.S.] 11: 473–504. 1917.
5. HOPKINS, J. C. F. Annual report of the Senior Plant Pathologist for the year ending 31st December, 1939. Rhodesian Agric. Jour. 37: 411–423. 1940.
6. LARSH, HOWARD W. Diseases observed on soybeans in Arkansas. U. S. Dept. Agr., Pl. Dis. Reptr. **28**: 956–957. 1944.

7. Leach, J. G.　The production of perithecia in *Ceratostomella ips* Rumb.　Phytopath. 24: 1037–1040.　1934.

8. Lehman, Samuel G.　Pod and stem blight of soybean.　Ann. Mo. Bot. Gard. 10: 11–178.　1923.

9. Luttrell, E. S.　Additional hosts of *Diaporthe sojae*.　U. S. Dept. Agr., Pl. Dis. Reptr. 29: 89–90.　1945.

10. Nitimargi, N. M.　Studies in the genera *Cytosporina*, *Phomopsis*, and *Diaporthe*. VIII.　Chemical factors influencing sporing characters.　Ann. Bot. 49: 19–40. 1935.

11. Pady, Stuart M.　Notes on the plant disease survey in Kansas, August to Nov., 1943.　U. S. Dept. Agr., Pl. Dis. Reptr. Suppl. 149: 313–316.　1944.

12. Person, L. H.　Report on diseases of soybeans in Mississippi (Sept. 27–Oct. 2). U. S. Dept. Agr., Pl. Dis. Reptr. 27: 509.　1943.

13. Sartoris, George B.　A cytological study of *Ceratostomella adiposum* (Butl.) comb. nov., the black rot fungus of sugar cane.　Jour. Agr. Res. [U.S.] 35: 577–585. 1927.

14. Seymour, Arthur Bliss.　Host index of the Fungi of North America.　732 pp. Harvard Univ. Press, Cambridge, Mass.　1929.

15. Shanor, Leland and Carlton F. Taylor.　*Diaporthe sojae* on cowpeas.　U. S. Dept. Agr., Pl. Dis. Reptr. 28: 81.　1944.

16. Tucker, C. M.　*Diaporthe phaseolorum* on pepper fruit.　Mycol. 27: 580–585.　1935.

17. Wehmeyer, L. E.　The genus *Diaporthe* Nitschke and its segregates.　X1 + 349 pp., 18 pl., Univ. Michigan Press, Ann Arbor, Mich.　1933.

18. Wolf, F. A., and S. G. Lehman.　Notes on new or little known plant diseases in North Carolina.　North Carolina Agr. Exp. Rept. 43: 55–58.　1920.

19. ————, and ————.　Diseases of soybeans which occur both in North Carolina and the Orient.　Jour. Agr. Res. [U.S.] 33: 391–396.　1926.

SEEDLING BLIGHT AND ROOT ROT OF FLAX IN WASHINGTON[1]

MAX L. SCHUSTER AND E. J. ANDERSON[2]

INTRODUCTION

Many complaints of poor stands of flax, even when planted under apparently favorable conditions, have been received from Washington growers. Moreover, workers in the Washington Agricultural Experiment Station, at Pullman, in their varietal yield trials found that samples of seed gave different emergence counts depending on the source of the seed. Workers in Europe and North America have shown that species of *Pythium, Phytophthora, Asterocystis, Ophiobolus, Helminthosporium, Botrytis, Alternaria, Cladosporium, Colletotrichum, Rhizoctonia*, and *Thielavia* were involved in the so-called "soil sickness" complex. The literature on the subject was reviewed by Schilling (4) and Tervet (6). Flor (1) studied *Fusarium oxysporum* f. *lini* (Bolley) Snyder and Hansen (*Fusarium lini* Bolley) and found this organism to be mainly responsible for soil sickness in North Dakota. It has been shown also that flax with injured seed coats gave poor emergence (2, 3, 5). Treatment of seed with protective dusts has improved stands, particularly when cracked seed was used (2, 3, 5). Seed treatment was of little value in North Dakota, since the wilt organism was primarily responsible for flax injury there (1). Investigations of the causes of poor stands of flax in Washington and of the effectiveness of seed treatments for the improvement of stands were initiated in 1941.

METHODS

To determine the organisms responsible for seed decay and seedling blight of flax, isolations were made from the surface of seed and from diseased roots and stems of seedlings from seed grown in Washington. Whole seeds and bits of diseased root and stem tissue were planted on potato-dextrose agar in Petri dishes in the usual manner. The resulting isolates which were to be tested for pathogenicity were purified by making successive transfers of hyphal tips, or of single spores where possible. Many isolates were eliminated after preliminary tests on the flax variety Bison. All isolates were identified as to genus. Those which proved pathogenic in preliminary tests were identified as to species. The fungi to be tested for pathogenicity were increased in Richard's solution in Erlenmeyer flasks and the mycelial mats were cut into small strips for addition to 4-inch pots of steamed soil. Twenty-five flax seeds were sown in each of these pots and each experiment was replicated four times in the greenhouse.

Determination of percentage of cracked seed and selection of cracked seed for study were done with the aid of a dissection microscope.

The value of seed treatments in improving stands was determined in the greenhouse for five varieties of flax, namely: Bison, Rio, Redwing, Viking, and Zenith.   Machine-threshed and hand-threshed lots of seed were treated with New Improved Ceresan (5 per cent ethyl mercuric phosphate), Micronized Sulphur, Semesan (30 per cent hydroxymercuri chlorophenol), Spergon (tetrachloro-parabenzoquinone), Du Bay 1205–FF (50 per cent tetramethyl thiuramdisulfide), Yellow Cuprocide (83 per cent yellow cuprous oxide), and Micronized Copper at the rate of 0.25 per cent by weight and planted in the greenhouse bench.   Stands were determined by an actual count of seedlings soon after the first true leaves unfolded.   Similar tests were made with the variety Bison, threshed by hand and by machine at low and high cylinder speeds,[3] planted in the field.

FUNGI ISOLATED FROM SEED AND SEEDLINGS

Fungi isolated from flax seed were species of *Penicillium, Alternaria, Aspergillus, Rhizopus,* and *Trichoderma.*   Of these *Penicillium spp.* were isolated most frequently.   Preliminary tests of these organisms in steamed soil failed to indicate marked pathogenicity for flax.   Cultures were retained for studies with cracked seed.

Fungi isolated from flax seedlings grown in the field at Pullman were principally *Fusarium spp.* and *Alternaria spp.*   *Fusarium* was isolated from such material 76 times while *Alternaria* was isolated only six times.   Preliminary study showed the isolates of *Fusarium* to be similar and pathogenic although some were more strongly pathogenic than others.   Isolates of *Alternaria* again were not pathogenic.

Isolation of fungi from diseased seedlings produced in pans of soil in the Seed Laboratory yielded *Fusarium* and *Rhizopus.*   *Rhizopus* proved nonpathogenic and was discarded.   Isolates of *Fusarium* were pathogenic and similar to those obtained from field-grown seedlings.

In the course of preliminary tests of the pathogenicity of isolates and

TABLE 1.—*Frequency of isolation and pathogenicity of most active isolates of Fusarium oxysporum and F. solani to flax in steamed soil as indicated by emergence and root and stem lesions on survivors*

Fungus	Isolates	Pathogenicity to flax	
		Emergence	Survivors with root and stem lesions
	Number	*Per cent*	*Per cent*
Fusarium oxysporum	159	27	70
F. solani	8	85	18
None		89	1

[3] This seed was obtained from Dr. O. A. Vogel, Agronomist, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture.

attendant comparison of colony characteristics and spores on potato-dextrose agar, it became evident that two pathogenic species had been obtained. These were identified as *Fusarium oxysporum* Schlecht and *F. solani* (Mart.) App. and Wr.[4] *F. oxysporum* was isolated from diseased seedlings 159 times while *F. solani* was isolated only eight times (Table 1). *F. oxysporum*, in later pathogenicity tests, also proved to be the more strongly pathogenic of the two. Differences in pathogenicity of three *Fusarium* isolates are shown in figure 1.

Isolates of *Fusarium oxysporum* obtained in other studies from roots of peas compared well in pathogenicity tests on flax with the isolates obtained from flax seedlings. Symptoms produced by this organism on flax consist of pre-emergence decay, damping-off, or, on older seedlings, pink to red or later brown, irregular lesions on root or stem, or both. These lesions may

Fig. 1. Emergence of Bison flax seedlings from 25 seeds sown in soil infested with 3 isolates of *Fusarium*. Check, at left, in steamed soil.

be small or involve the entire root and the base of the stem. Tests of the fungus on the wilt-susceptible variety Punjab failed to show yellowing, thickening of apical leaves, or wilting, characteristic of flax wilt, among the survivors. Symptoms of affected seedlings were alike in field soil and in pots of steamed soil to which *F. oxysporum* was added, but the incidence was higher in the latter.

SEED COAT INJURY AS A FACTOR IN SUSCEPTIBILITY TO FUNGUS ATTACK

Other workers have shown that sample lots of flax seed vary in amount of cracking. Machacek and Brown (2) found more cracking in samples from Western than from Eastern Canada. These workers also state that large-seeded varieties seemed more susceptible to injury than the small-seeded lots. Moore and Christensen (3) found that seed of Bolley's Golden, selection C.I. 976, grown in 31 localities in North America varied from 0 to 32 per cent cracked seed. They stated "the differences in amount of injury between seven varieties in a single locality were sometimes almost as great

TABLE 2.—*Percentage of cracked seed in flax varieties grown in Washington*[a]

Variety	Number of seed examined	Percentage of seed cracked
Bison	123	75
Viking	124	65
Rio	232	42
Redwing	189	15
Zenith	336	10

[a] Viking from 1940 crop; all others from 1941 crop.

as those between seed lots of a single variety grown at different localities."
The fact that a sample of Bison flax from Minnesota gave better stands than
a sample of Bison from the Palouse, although markedly pathogenic organ-
isms were not present, made it seem reasonable to assume that cracked seed
coats were responsible in part for flax seedling diseases in the Palouse.

In seed lots of several flax varieties grown in Washington 65 to 75 per
cent of the seeds had at least slight cracks in the seed coat, as determined
by examination under a dissecting microscope. In some cases as much as
50 per cent were severely damaged. Present data show that while there is a
tendency for large-seeded varieties to be more severely damaged, size alone
does not determine the degree of damage. The variety Rio, which has
larger seeds than Bison, had considerably less cracking (Table 2). Severe
cracking is not confined to the seed coat, but extends into the seed and fre-
quently damages the embryo. The cotyledons may be broken or the radicle
injured so as to prevent proper germination. Machacek and Brown (2),
and Moore and Christensen (3), have shown that there is a relationship
between cracking in a seed lot and percentage stand obtained.

Comparative tests were made of the attack on cracked and sound seed
by isolates 1 and 2 of *Fusarium* and by the more common seed-borne organ-
isms, *Penicillium* and *Alternaria* (Table 3). *Fusarium* isolate 2 was equally
virulent on cracked and sound seed. The *Fusarium* isolate 1 was pathogenic
to cracked seed but not to sound seed. These isolates were similar in culture
and identified as *F. oxysporum*. No further studies of strain differences
were made. *Penicillium* and *Alternaria*, though detrimental, were much
less so than the Fusaria. The data definitely show that fungi ordinarily

TABLE 3.—*Seedling emergence from cracked and sound flax seed inoculated with
isolates of Fusarium, Penicillium, and Alternaria*

Fungus	Number of plants emerged[a] from seeds with seed coat	
	Sound	Cracked
Fusarium oxysporum 1	94	54
F. oxysporum 2	35	32
Penicillium sp.	93	70
Alternaria sp.	94	76
None	95	88

[a] Mean of two tests with 100 seeds in each variation.

FIG. 2. Effect of broken seed coats of flax on pathogenicity of the otherwise avirulent *Fusarium* isolate 1. Pot A, sown with sound, uncracked seed and not inoculated; B, with cracked seed, not inoculated; C, with sound, uncracked seed, inoculated; D, with cracked seed, inoculated.
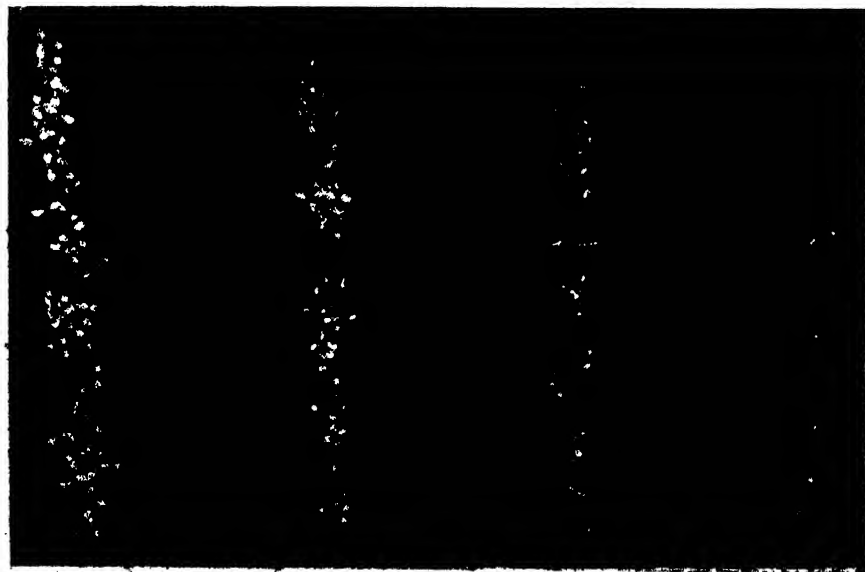
FIG. 3. Response of uninjured and injured flax seed to treatment with Spergon, in the greenhouse. Left to right—sound seed, treated; sound seed, not treated; cracked seed, treated; cracked seed, not treated.

considered as saprophytes can cause severe losses when seed-coats are injured. The influence of cracked seed is shown also in figures 2 and 3.

CONTROL OF DAMPING-OFF AND SEEDLING BLIGHT

Seed treatment has been a satisfactory means of controlling damping-off in Washington (5), Minnesota (3), and Canada (2). Seed of six flax varieties was threshed by hand or by machine at high cylinder speed. Seven lots treated with different fungicides and one check lot were sown in the greenhouse in 1942 (Table 4). The stands were greater for untreated hand

TABLE 4.—*Effect of seed treatment, applied at the rate of 0.25 per cent by weight, on emergence of flax seed threshed by hand and by machine at high cylinder speed. Greenhouse, 1942*

Variety	Type of thresh-ing	Check[a]	Percentage increase in stand over check due to treatment with						
			New Improved Ceresan	Micro-nized sulphur	Semesan	Spergon	Du Bay 1205-FF	Yellow Cuprocide	Micro-nized per
Bison	Hand	91	27	24	23	23	13	13	20
	Machine	20	360	– 35	350	430	480	145	260
Redwing	Hand	96	22	27	16	25	13	19	25
	Machine	52	54	– 12	110	87	88	83	81
Zenith	Hand	96	– 3	0	13	9	23	16	– 2
	Machine	48	102	8	110	83	113	78	106
No. 1046	Hand	95	– 24	– 3	– 13	– 5	– 14	2	– 17
	Machine	55	– 24	– 5	64	65	58	49	36
Viking	Hand	72	25	1	33	31	39	31	18
	Machine	19	26	111	275	442	400	153	146
Rio	Hand	97	23	15	– 7	9	– 1	4	15
	Machine	42	29	174	88	126	150	95	50

[a] The numbers indicate means of actual stands in four replications of 100 seeds.

threshed seed of all varieties than for machine threshed. This appears to confirm the statement that cracking is due chiefly to threshing, as reported by workers in Minnesota (3) and Canada (2). Furthermore, the machine threshed seed lots responded better to seed treatment than did hand threshed lots (Fig. 3). In general, the average increase of stand over the check for all varities and treatments, was 12 per cent for hand threshed and 120 per cent for machine threshed. This corroborates the findings of workers in Minnesota (3) and Canada (2) that injured seed gave greater response to treatment than sound seed. Spergon, Semesan, and Du Bay 1205-FF were equally effective in increasing stand of machine-threshed seed and were more effective than New Improved Ceresan.

Seed treatments were tested in the field in 1943, with Bison flax treated with the several chemical compounds. In addition to the seed from the lots used in the greenhouse trials, a seed lot threshed by machine run at medium cylinder speed was included. The results are summarized in the analysis

TABLE 5.—*Analysis of variance of emergence of flax in the field trials. Seed treatment experiment, 1943*

Source of variation	Degrees of freedom	Mean Square	F	F at 1 per cent point
Threshing treatments	2	5820	113.00**	4.88
Chemical treatments	6	199	3.86**	3.04
Treatment interaction	12	33	0.64	2.41
Error	80	52	.	

** Indicates significance.

of variance presented in table 5. Treatment of seed with fungicides was highly significant. The compounds, Du Bay 1205–AK (tetramethyl thiuramdisulfide), Spergon, and New Improved Ceresan, used in this experiment were equally effective in protecting the seed. Similarly, emergence as related to threshing methods is highly significant. The average emergence per row for untreated seed threshed by hand, slow machine, and fast machine was 87, 76, and 54 plants, respectively.

<div align="center">DISCUSSION</div>

The foregoing studies show that *Fusarium oxysporum* causes damping-off and seedling blight of flax in Washington. *Fusarium oxysporum* f. *lini* (*Fusarium lini*) is the only *Fusarium* heretofore reported to attack flax, and this species causes wilt as well as seedling blight. Flax wilt has not been found in Washington.

Certain organisms generally considered saprophytic, both seed- and soil-borne, also have been shown to be important enemies of successful flax culture in Washington. Such omnipresent fungi as *Penicillium* and *Alternaria* can reduce stands when seeds with injured coats are sown, but are harmless to sound seed.

Experiments in which seeds were threshed by different methods show a variation in emergence of flax. Hand-threshed seed gave good stands in contrast to machine-threshed lots. Seed threshed by machine at a medium cylinder speed gave better stands than did seed threshed at a high cylinder speed. Apparently damage is due chiefly to mechanical injury, although as reported by Moore and Christensen (3) natural injury has been found.

Flax stands were improved by the use of fungicides such as Spergon, Du Bay 1205–FF, DuBay 1205–AK, Semesan, and New Improved Ceresan. Seed with a greater amount of injury responded to a greater degree to seed treatment. It is assumed that the cracks afford soil-borne organisms ready access to the interior of the seed before the seedling has time to become established. The damage to the seed also may permit loss of moisture and subsequent death to the embryo, so that stands may be poor regardless of seed treatment. Furthermore, these injured areas would seem to be ideal places for seed-borne organisms to lodge and later cause damping-off.

<div align="center">SUMMARY</div>

1. *Fusarium oxysporum* causes damping-off and seedling blight of flax in Washington.

2. Species of *Penicillium* and *Alternaria* reduce stands of flax by attacking seed with broken testas.

3. In five flax varieties studied, the percentage of cracked seed was 10–15 for Zenith and Redwing, 65–75 for Viking and Bison, and 42 for Rio.

4. Seed threshed by hand gave better stands in field and greenhouse than seed threshed by machine at medium cylinder speed. Seed threshed by machine at high cylinder speed gave the poorest stands.

5. Stands of flax in field and greenhouse were improved by the use of Spergon, Du Bay 1205–AK, Du Bay 1205–FF, and New Improved Ceresan.

WASHINGTON STATE COLLEGE,
    PULLMAN, WASHINGTON.

LITERATURE CITED

1. FLOR, H. H.   Soil sickness of flax in North Dakota.   Phytopath. **30**: 749–750.   1940.
2. MACHACEK, J. E., and A. M. BROWN.   Preliminary investigations on mechanical injury in flax seed.   Phytopath. **32**: 733–734.   1942.
3. MOORE, M. B., and J. J. CHRISTENSEN.   Natural and mechanical injury in flax in relation to seed treatment.   (Abstr.) Phytopath. **33**: 1117.   1943.
4. SCHILLING, E.   Krankheiten und Beschädigungen des Flachses.   In Tobler, Fr., Der Flachs als Faser- und Ölpflanze.   273 pp.   Berlin.   1928.
5. SCHUSTER, MAX L., DWIGHT D. FORSYTH, and J. G. HARRAR.   Influence of certain diseases and threshing injury on flax stands in Washington.   Washington Agricultural Experiment Station "V" Circ. 8.   1943.
6. TERVET, IAN W.   An experimental study of some fungi injurious to seedling flax.   Phytopath. **27**: 531–546.   1927.

THE ZINC SALT OF 2,4,5-TRICHLOROPHENOL[1] AS A SEED FUNGICIDE

L. J. MEULI, B. J. THIEGS, AND G. E. LYNN

A large number of phenols and phenol derivatives have been tested as agricultural fungicides. Although many of them have good fungicidal properties, most are highly injurious to growing plants. However, the fact that seeds are not so readily injured by chemicals suggested research on several phenols as seed protectants.

MATERIALS AND METHODS

The chemicals to be tested were prepared by mixing them with talc and grinding the mixture to fine dusts in a hammermill. The resulting dusts, measured by Gooden and Smith's[2] air permeation method, had average particle sizes ranging from 4 to 8 microns.

All cracked, discolored, and off-sized seeds were removed from seed lots to be used. Weighed amounts of the culled seed, generally 500 grams, were treated, the dust dosages being reported in the results as grams per kilogram of seed.

Mixing of seeds and dusts was accomplished by rotating in round-bottom flasks for three minutes by means of an electrically driven apparatus.

All seeds, with the exception of cotton, were grown in soil composed of 2 parts muck, 2 parts black loam, and 1⅓ parts sand. It was established that this soil mixture contained pathogens such as *Rhizoctonia*, *Pythium*, and certain *Fusarium* which actively reduce seedling stands. In occasional tests macerated cultures of *Rhizoctonia solani* Kühn were added to supplement the pathogenic fungi contained in the greenhouse soil.

A higher incidence of damping-off of peas was obtained when the seeds were subjected to a short pre-cooling period which simulated cool spring temperatures so frequently favorable for optimum disease conditions in the field. The seeds were planted in 12-ounce paper pots containing greenhouse soil; the pots were uniformly watered and stored one week at 8.0° C. before they were placed in the greenhouse. Seeds not requiring cold storage were sown in flats of soil and were placed directly in the greenhouse. Cotton seed, bearing natural inoculum of the anthracnose fungus (*Colletotrichum gossypii* South., known in the perfect stage as *Glomerella gossypii* (South.) Edg.) on their surface, were sown in flats of washed sand in the greenhouse held at approximately 23° C. To supplement all tests run in the greenhouse an identical series of treated and untreated seeds was planted in clean sand in order to ascertain whether or not the chemical treatment retarded or reduced seed germination.

Records of seedling survival and disease-free seedlings were made after seven to fourteen days growth in the greenhouse.

RESULTS

The results obtained in a representative test with a series of chlorophenols applied as dusts at the rate of 2 grams per kilogram of seeds of Alaska pea, Henderson lima bean, and Virginia bunch peanut (machine shelled), and 4 grams per kilogram of fuzzy cotton (Delfos No. 651) are in table 1. The percentage of clean seedlings recorded is based on 200 seeds planted per treatment.

TABLE 1.—*The effect of 50 per cent chlorophenol seed dusts on seedling survival*

Chemical treatment	Percentage disease-free seedlings survived			
	Pea	Cotton[a]	Lima bean	Peanut
Phenol	63	34	48	22
2-Chlorophenol	62	3	54	16
4-Chlorophenol	54	23	43	22
2,4-Dichlorophenol	79	34	50	28
2,4,5-Trichlorophenol	86	32	70	75
2,4,5-Trichlorophenol, Na salt	86	62	74	66
2,4,6-Trichlorophenol	69	24	72	65
2,4,6-Trichlorophenol, Na salt	77	42	54	73
2,3,4,6-Tetrachlorophenol	74	58	68	69
2,3,4,6-Tetrachlorophenol, Na salt	79	59	66	64
Pentachlorophenol	41[b]	47	32[b]	67
Pentachlorophenol, Na salt	48[b]	60	30[b]	60
Untreated	71	4	35	24

[a] Seed naturally infested with *Colletotrichum gossypii*.
[b] Growth retarded.

Phenol was included in this series as a compound having known germicidal qualities. It was but slightly effective as a seed fungicide at the concentration used. This was also true of the mono and dichlorophenols. Similar results not included in these data have been obtained with other mono and dichlorophenols tested.

Most consistently effective in producing healthy seedlings were 2,4,5-trichlorophenol and its sodium salt, although the parent 2,4,5-trichlorophenol was but slightly effective on cotton seed. The 2,4,6-trichlorophenol and its sodium salt were slightly less effective than the 2,4,5-trichlorophenols, except on lima bean and on peanuts. Tetrachlorophenol and its sodium salt were generally effective but not so active fungicidally as the 2,4,5-trichlorophenols on all seed types. Pentachlorophenol and its sodium salt were no better than the mono and dichlorophenols; and, in a few instances, chemical retardation of seedling growth was observed in both the sand and soil cultures.

Those phenols having good activity were compounded with talc to give dusts of several concentrations and applied at the rate of 2 grams of the

dust composition per kilogram of Alaska pea seed. Seedling survivals based on 400 seeds planted per treatment are given in table 2.

The data indicate the superiority of the 2,4,5-trichlorophenol and its sodium salt over the other chemicals tested at all concentrations, the effectiveness increasing with the concentration. The low survival of seedlings in most of the treatments is due to the rigorousness of this experiment in which macerated cultures of *Rhizoctonia solani* supplemented those organisms contained in regular greenhouse soil. This is well illustrated by the low survival figure for the untreated seeds. Germination tests in washed sand showed chemical injury or retardation of germination to be severe where the seeds were treated with 50 per cent pentachlorophenol, slight with 50 per cent 2,3,4,6-tetrachlorophenol, and none with 50 per cent 2,4,5-trichlorophenol. Some increase in seedling survival was obtained with the

TABLE 2.—*Effect of different concentrations of chlorophenol dusts on seedling survival of Alaska pea*

Chemical treatment	Percentage of seedling survival of pea at chemical concentrations of:						
	10 per cent	20 per cent	35 per cent	50 per cent	65 per cent	80 per cent	95 per cent
2,4,5-Trichlorophenol	8	10	19	22	27	28	22
2,4,5-Trichlorophenol, Na salt	11	11	19	29	20	39	51
2,4,6-Trichlorophenol	7	6	8	8	....	.... .	......
2,3,4,6-Trichlorophenol	0	0	0	0	....	....	......
2,3,4,6-Tetrachlorophenol, Na salt	0	1	0	0	.. ..	.... .	......
Untreated	2	2	2	2	....	.... ..	......

concentrations higher than 50 per cent but the dust compositions were physically unsatisfactory. Normal germination of the peas was obtained in washed sand even at the three highest concentrations of 2,4,5-trichlorophenol and its sodium salt.

Continued investigations with varied dust concentrations and dosages showed that the sodium salt of 2,4,5-trichlorophenol was superior to the parent 2,4,5-trichlorophenol as a seed treatment fungicide. The sodium salt of 2,4,5-trichlorophenol was especially effective on cotton seed; its volatile action appeared to have some value as a disinfectant for the control of the seed-borne anthracnose pathogen as well as acting as a protectant against soil-inhabiting organisms active in pre-emergence killing. The efficacy of the sodium salt over the parent phenol indicated that other metal salts should be tried.

Nine metal salts of 2,4,5-trichlorophenol were prepared as dust compositions, each containing 50 per cent of the active ingredient. Alaska peas, 200 per treatment, were dusted with these materials at the rate of 2 grams per kilogram of seed and planted in soil containing damping-off organisms. Seedling survival records (Table 3) from greenhouse tests showed that the zinc, lead, copper, potassium, and barium salts were significantly better

TABLE 3.—*Effect of metal salts of 2,4,5-trichlorophenol (50 per cent active ingredient) on seedling survival of Alaska pea seeds*

Salts of 2,4,5-trichlorophenol	Percentage seedling survival
Zinc	93.5
Lead	92.5
Copper	89.5
Potassium	87.5
Barium	84.5
Sodium	80.5
Mercuric	78.0
Silver	67.5
Calcium	67.5
Untreated	74.5
Least significant difference between means (0.01 level)	3.5

than the sodium salt in the order named. All treatments, except the silver and calcium salts of 2,4,5-trichlorophenol, increased the seedling survival significantly over the checks.

In table 4 are shown representative greenhouse results of several of the best metal salt compositions compared with the sodium salt of 2,4,5-trichlorophenol applied as dusts to several types of agricultural seed, using 300 seeds per treatment.

Treatment with zinc salt of 2,4,5-trichlorophenol consistently resulted in high seedling survival for all seeds treated. Of particular note is the fact that this compound also controlled the seed-borne *Colletotrichum gossypii*. Only in the case of Detroit red beet and Virginia Savoy spinach seed treatment was the zinc salt excelled by the copper salt, although these differences may not be significant.

Seeds treated with different concentrations and dosages of zinc salt of 2,4,5-trichlorophenol were planted in the greenhouse. In table 5 are repre-

TABLE 4.—*Comparison of metal salts of 2,4,5-trichlorophenol (50 per cent active ingredient) as seed fungicides*

	Percentage seedling survival					
	Dosage in grams of dust composition per kg. of seed					
Salt	4	2	2	2	2	12
	Fuzzy cotton[a]	Lima bean	Peanut	Red beet	Spinach	Lettuce
Zinc	81	62	56	150[b]	87	85
Lead	71	60	42	123[b]	86	79
Copper	45	44		168[b]	88	73
Sodium	81	34	17	99	63	75
Untreated	65	24	2	118[b]	72	77

[a] Seed naturally infested with *Colletotrichum gossypii*.

[b] Emergence figures over 100 per cent due to presence of several viable germs for each seed ball.

sentative data from cotton seed tests sown immediately after treating and 4 weeks after treating with several concentrations and dosages of zinc salt of 2,4,5-trichlorophenol. Effectiveness of the material was maintained over the 4-week period between treating and testing. From these and similar tests it was concluded that the optimum concentration of the zinc salt of 2,4,5-trichlorophenol was near the 50 per cent dust composition applied at a dosage of 3 grams per kilogram. Higher concentrations were not so easily handled and seedling emergence was reduced at higher dosages. The lowest concentration was not so effective except at the highest rate of application.

TABLE 5.—*Effect of concentration and dosage of the zinc salt of 2,4,5-trichlorophenol on seedling survival of fuzzy cotton seed*[a]

| Concentration (in per cent) of Zn-trichlorophenate | Dosage, gm./kg. | Percentage disease-free cotton seedlings survived | |
		Test 1[b]	Test 2[b]
100	1.5	49	65
	3.0	63	67
	6.0	47	54
	12.0	20	35
75	1.5	55	60
	3.0	52	56
	6.0	61	65
	12.0	33	44
50	1.5	49	50
	3.0	47	67
	6.0	53	66
	12.0	40	62
25	1.5	36	39
	3.0	45	43
	6.0	54	48
	12.0	51	66
Untreated	...	8	2

[a] Seed naturally infested with *Colletotrichum gossypii*.
[b] Test 1 sown immediately after treatment.
Test 2 sown four weeks after treatment.

In table 6 are shown the results of additional greenhouse tests with dust concentrations containing 10 to 50 per cent zinc salt of 2,4,5-trichlorophenol on Alaska pea, reginned cotton seed (Mexican Big Boll), and fuzzy cotton seed (Delfos No. 651).

On pea seed, the greatest fungicidal effectiveness was obtained with the highest concentration and dosage used. There was no indication of injury from any of the treatments.

On reginned cotton seed the differences between treatments were not so great as differences among pea treatments but more consistent results were obtained with the highest concentrations. The large number of disease-free seedlings from the untreated seed indicated that this heavily reginned seed had lost much of its spore inoculum in the removal of seed fibers.

With the Delfos fuzzy seed, which carried a high spore load on the seed

fibers, the results indicated that all four concentrations of the zinc salt of 2,4,5-trichlorophenol were effective when applied at 3 grams per kilogram of seed.   More exacting field studies should prove which concentration is most desirable.

TABLE 6.—*Effect of concentration and dosage of the zinc salt of 2,4,5-trichlorophenol on seedling survival*

Concentration (in per cent) of zinc salt of 2,4,5-trichlorophenol	Dosage, gm./kg.	Percentage disease-free seedlings survived		
		Alaska pea	Reginned Cotton[a]	Fuzzy cotton[a]
10	2.0	40.5	63.0	... ...
	4.0	49.5	63.0	. .
	6.0	50.0	..	... .
20	1.5	....	....	56.5
	2.0	52.5	58.8	..
	3.0	... ...	.	75.0
	4.0	70.0	70.5	..... .
	6.0	73.0	......	. ....
30	1.5	... ..	.. ..	69.5
	2.0	47.0	65.5	.
	3.0	... .	.	77.0
	4.0	65.0	80.0	. .
	6.0	82.0	... .	...
40	1.5	.... .	.... ..	71.0
	2.0	63.5	71.3	.
	3.0	... .	.... .	79.0
	4.0	79.0	71.5	
	6.0	90.5	.. ..	. ..
50	1.5	.... ..	....	77.5
	2.0	67.5	68.0	. .
	3.0	......	. ...	77.0
	4.0	89.0	75.0	... ...
	6.0	96.5	.. ...	... .
Untreated	....	24.2	55.2	13.0
Least significant difference between means (0.01 level)		2.8	4.2	19.1

[a] Seed naturally infested with *Colletotrichum gossypii*.

DISCUSSION

The results given in this paper indicate that the zinc salt of 2,4,5-trichlorophenol is the most effective of the phenols tested as a fungicide for seed treatment.   Preliminary tests with other phenol derivatives, not reported here, also indicated that the zinc salt of 2,4,5-trichlorophenol is the most active phenol fungicide for the greatest number of economic seeds tried. Considerable work remains to be done before optimum dilution of the active chemical and dosages of the dust can be suggested for seeds of specific plants. It is generally conceded among investigators of seed fungicides that no universal seed treatment is likely to be found and that no general recommendations can be made on the basis of results obtained on a few species.

The comparison of the metal salts of 2,4,5-trichlorophenol in table 4 pro-

vides an interesting illustration of variation in fungicidal activity of closely related compounds. For example, the sodium salt was approximately equal in activity to the zinc salt for cottonseed treatment, but it was less active on the other seed types.

SUMMARY AND CONCLUSIONS

The zinc salt of 2,4,5-trichlorophenol has been very effective as a fungicide for seed treatment. It was superior to other phenol derivatives tested.

The most effective concentration range of the active ingredient for seed treatment preparations appears to be 30 to 50 per cent of zinc salt of 2,4,5-trichlorophenol combined with an inert diluent. Dosages have been established for seeds of only a few species, but it appears that the usual dosage recommendation of 2 to 4 grams per kilogram for large seeds like cotton may be generally applicable.

In addition to reducing pre-emergence killing of seedlings by soil-inhabiting fungi, the zinc salt of 2,4,5-trichlorophenol has also been effective in reducing seedling infection by *Colletotrichum gossypii* South. when seed naturally infected by this fungus is treated before planting.

THE BIOCHEMICAL RESEARCH LABORATORY,
  THE DOW CHEMICAL COMPANY,
    MIDLAND, MICHIGAN.

MORPHOLOGY AND THE MODE OF TRANSMISSION OF THE RAGI SMUT

M. J. THIRUMALACHAR AND B. B. MUNDKUR

Ragi or nachni (*Eleusine coracana* Gaertn. f.) is an important cereal grown in Mysore and in parts of the Bombay and Madras Provinces (India). In Mysore a large number of people use it for food, and extensive tracts of land are therefore under ragi cultivation. Very few diseases due to fungi are known to attack this crop but a smut due to *Melanopsichium eleusinis* (Kulkarni) Mundkur and Thirumalachar, which often does damage, was first described by Kulkarni (3) on material collected by him at Malkapur in the Kolhapur State. He named the fungus *Ustilago eleusinis*.

The mode of transmission of this smut and methods of controlling it are, however, still unknown. Kulkarni (3) states that he smeared viable spores on ragi seeds which he sowed in pots, and he claims that he obtained smutted ears in the resulting crop. Field experiments by McRae (4) did not, however, corroborate Kulkarni's findings. A more detailed investigation on the cytology of the fungus and its mode of transmission was, therefore, considered highly desirable. Pot experiments were conducted at Bangalore at the proper ragi sowing time and the results obtained are presented.[1]

DESCRIPTION OF THE SMUT

In Mysore two crops of ragi are raised in a year. The "kar" ragi is sown in February–March and harvested in June–July, the crop being cultivated in moist places following the rice crop. There is practically no smut on this crop. The second crop, known as the "hain" crop, is sown in July–August and harvested in November–December. Much damage is caused, however, to this crop by smut. The smut is first evident some time after flowering has started and is, as a rule, scattered at random in the ear (Fig. 1, 1) about five to six grains out of nearly 200 being affected. When the disease occasionally appears in an epidemic form as many as 15 grains in an ear may be attacked. The diseased grains are transformed into galls or bullate bodies, six to seven times the normal size of the grain. In the early stages of attack the affected grains are slightly greenish, and 2–3 mm. in diameter; and they project slightly beyond the glumes. As development proceeds, the attacked grains swell and reach a diameter up to 16 mm. The greenish outer tunica of the sorus gradually turns pinkish green and shows signs of rupturing at several places.

Dissection of the infected spikelet at various stages of development and a study of microtome sections of young sori reveal that the smut is strictly

ovariicolous. The short style, the feathery stigma, the stamens, and the glumes are entirely unaffected (Fig. 1, 2 and 3).

CYTOLOGY OF DEVELOPMENT

Material for cytological study was collected at various stages of development in fields near Bangalore and fixed in formalin-acetic-alcohol or in Karpechenko's modification of Nawaschin's fluid. Microtome sections of 6
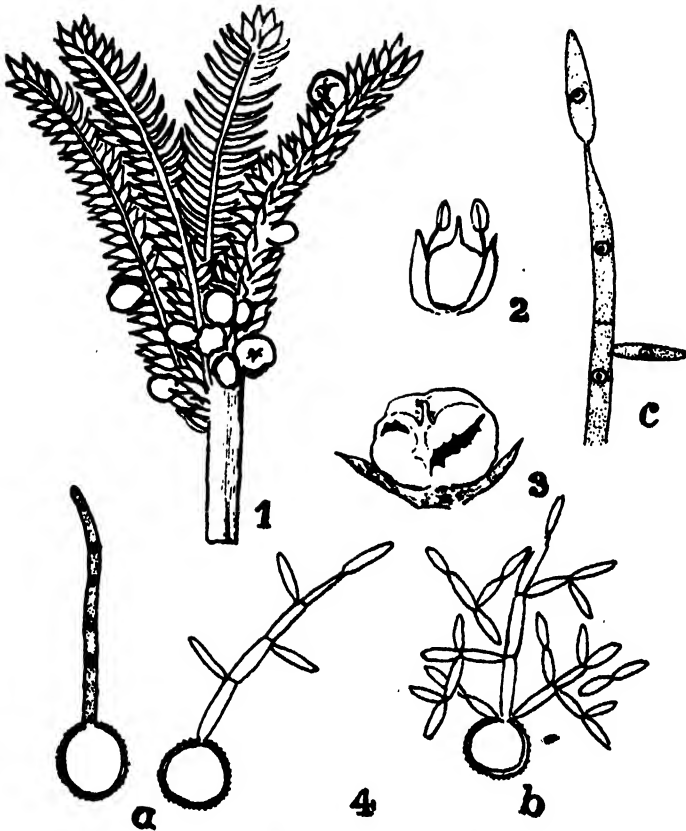
FIG. 1. The ragi smut. 1. A smutted ear of ragi. Natural size. 2. A young sorus × 2. 3. A nearly mature sorus × 3. 4. Stages in the germination of spores. a and b, × 750; c, × 1250.

to 10 μ thickness were cut and stained with Newton's iodine gentian violet or Haidenhain's iron alum haematoxylin with orange G as counter stain. The chlamydospores were germinated and then stained using the method suggested by Thirumalachar (8).

Sections through young sori show that in the initial stages of infection the ovary becomes multi-layered by the rapid multiplication of the cells. Immediately thereafter small lysigenous cavities appear at different parts of the ovary by the disintegration of the host cells. Gradually the cavities enlarge and become ovate or spherical. A thick felt of mycelium then

begins to border the cavities which become filled with a mucilaginous fluid that can be stained with Congo red or eosin B.

Chlamydospores form very soon thereafter from the mycelia bordering the cavities. Their development is thus centripetal, the oldest spores being in the center of the sorus. Young spores are somewhat polyhedral and thin-walled; mature spores are subglobose to spherical, yellowish-brown and minutely verruculose. They measure 7 to 11 μ in diameter with a mean of 9.6 μ. Due to the formation of two, three, or even four lysigenous cavities filled with these spores within a single swollen ovary, the sorus is locular, each locule being clearly and distinctly separated from the other by the host tissue (Fig. 2). Such locular sori are the distinguishing feature of the
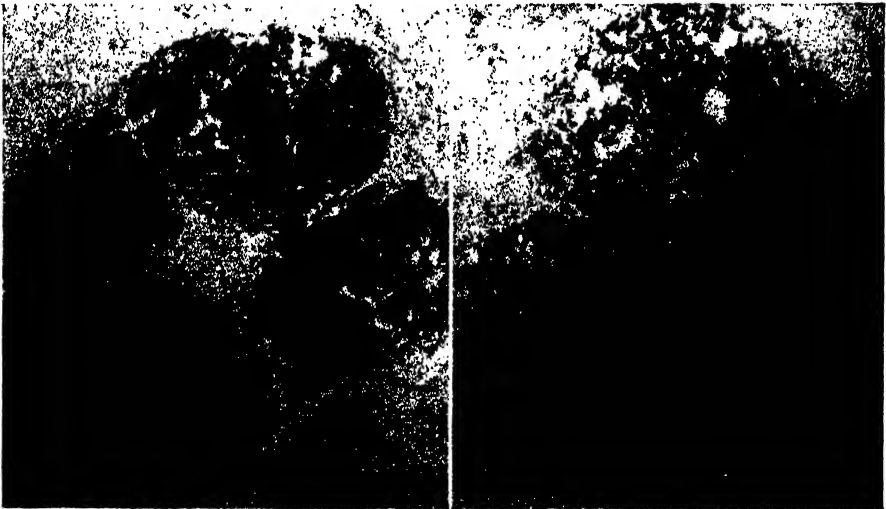
FIG. 2. Photomicrographs of the ovaries to show locular sori.

genus *Melanopsichium* Beck; and the ragi smut sori bear close resemblance to the sori of *Melanopsichium pennsylvanicum* Hirsch., one of the gall-forming smuts found on *Polygonum glabrum* in India. In this latter smut the masses of chlamydospores lie embedded in mucilage and later form tendril-like crusts because of the hardening of the mucilage. The mucilage in the sori of the ragi smut is not so copious or appreciable in quantity. All these characters led Mundkur and Thirumalachar (7) to rename the ragi fungus *Melanopsichium eleusinis*.

After the development of the chlamydospores the tissues of the ovary wall dry up and later, due to desiccation, the locular nature of the sorus becomes less conspicuous. The interior of the sorus then disintegrates and becomes a dusty mass of spores. The loose spores may be dispersed by wind but in many cases the outer rind of the host tissue covering the sorus remains persistent and the entire bullate sorus is detached from the panicle and drops to the ground. Later, as a result of the disintegration of the sorus, the spores are dispersed.

The spores germinate, without a rest period, when floated on rain water or nutrient solutions. For studying the nuclear details of germinating spores, permanent preparations were made. The promycelium first protrudes as a small papilla and then elongates into a stout germ tube. Following nuclear divisions, it becomes three-septate and bears both terminal and lateral sporidia (Fig. 1, 4). Mature sporidia are ovate to cylindric, uninucleate, thin-walled, and biguttulate. In spite of careful examination of several preparations sporidial conjugations have not been seen.

Chlamydospores germinate on potato-dextrose agar and form small white colonies within 3 or 4 days. Very little mycelium is produced in culture, but secondary sporidia are produced rapidly.

<div align="center">INOCULATION EXPERIMENTS</div>

Inoculation experiments were undertaken to determine the mode of transmission of the disease.

Inoculation of seed. Two separate tests were carried out. In one set, the seed was smeared with viable chlamydospores and sown in pots. In another, the seed was smeared with sporidia from a culture and then sown. For each set of experiments ten pots were used, with five plants per pot. Experiments were carried out at Bangalore. Adequate controls were provided.

Neither in the controls nor in the pots sown with inoculated seed did the disease appear. The experiments were repeated during two more seasons, but negative results were obtained every time.

Inoculation of seedlings. In some plants that were about three to five inches in height, sporidial suspensions were injected with a delicate hypodermic needle, but no chlorotic spots or smutted grains appeared in the plants.

Floral inoculation. When it became evident that the disease is not externally seed-borne, attempts were made to inoculate the flowers at the anthesis stage using the technique suggested by Moore (5) for inoculating wheat with the loose-smut fungus. Both viable spores and sporidial suspensions obtained from the culture medium were used. As the spores do not, on germination, form promycelial infection threads but only sporidia, it was thought unlikely that the fungus would form dormant mycelium within the seed.

The sporidial suspension was well dispersed within the glumes and pervaded the entire spikelets. The inoculated ears were enclosed in paper bags and kept under close observation. None of the grains, however, became infected. The seed was preserved and sown the next year in pots to see if smut would develop in the progeny. No such smut was noticed.

It was then surmised that the maturity of the flowers at the time of inoculation had some bearing on the capacity of the sporidia to infect. Microtome sections of the spikelets with smut sori had shown that at the time the sori contained mature spores, the noninfected ovule was at the

megaspore mother-cell stage, indicating that infection must have taken place at a very early stage of development of the spikelets.   Field observations had also shown that sori were present in ears that had just emerged from the sheaths.

Young ears that were still enclosed within the sheath were then selected for the inoculation experiments.   The inoculum, which consisted of suspensions of sporidia or chlamydospores, was dropped into 125 such sheaths in the form of a fine jet as suggested by Eddins (2).   The inoculated sheaths were often wrapped in moist cotton pads to provide moisture.   Adequate controls (20 sheaths) were treated in the same manner but without spores or sporidia.

After the ears had emerged from the sheaths, the cotton pads were removed.   When the ears matured, it was found that this method of inoculation was successful.   Out of 125 ears that were inoculated 17 were infected, but in each ear not more than 9, and in one case 12, grains were smutted. There was no smut in ears of the control plants.

These experiments indicate that early floral infection occurs in the ragi smut and that the disease is not carried in or on the seed.   Observations on the occurrence of smut sori in the ear and on the rapid production of sporidia rather than infection threads by the germinating chlamydospores support the view that the fungus is not systemic in ragi.   It seems probable that ragi is infected by air-borne inoculum just as wheat is infected by the karnal bunt *Neovossia indica* (Mitra) Mundkur (6) and as bajra (*Pennisetum typhoides*) is infected by *Tolyposporium penicillariae* Bref. (1).

<center>DISCUSSION</center>

These investigations indicate that ragi smut is probably an air-borne disease and that it is not carried in or on the seed.   This finding has made the problem of controlling ragi smut rather difficult.   Treatment of seed either with fungicidal dressings or by hot water is out of the question.   In efforts to discover resistant varieties there appears to be a good deal of hope, but that is a time consuming process.   Removal of smutted ears, which can be readily seen in the fields, so as to prevent the sori from lodging in the ground and deep ploughing to bury the sori so as to render them innocuous are perhaps some of the other methods that merit a trial.

<center>SUMMARY</center>

Ragi smut due to *Melanopsichium eleusinis* (Kulkarni) Mundkur and Thirumalachar is distributed in the ragi-growing areas of Mysore, Bombay, and Madras Provinces.

Sections of very young sori which are ovariicolous indicate that the fungus forms locular sori in lysigenous cavities.   The sorus converts the ovary into a large gall six to seven times the normal size of the grain.   Spores are formed centripetally.   They germinate readily producing primary and secondary sporidia.   Conjugation of sporidia has not been observed.

Several experiments to determine if the smut is externally seed-borne gave consistently negative results. When ears that were just emerging from the sheath were inoculated with sporidial or spore suspensions, about 13 per cent of the plants became smutted. These results indicate that floral infection takes place at an early stage and the smut is probably air-borne.

CENTRAL COLLEGE, BANGALORE,

AND

DIVISION OF MYCOLOGY AND PLANT PATHOLOGY,

INDIAN AGRICULTURAL RESEARCH INSTITUTE,

NEW DELHI, INDIA.

LITERATURE CITED

1. AJREKAR, S. L., and V. N. LIKHITE. Observations on *Tolyposporium penicillariae* Bref. (the bajra smut fungus). Curr. Sci. 1: 215. 1933.
2. EDDINS, A. H. Pathogenicity and cultural behavior of *Ustilago zeae* (Beckm.) Ung. from different localities. Phytopath. 19: 885–916. 1929.
3. KULKARNI, G. S. The smut of nachni or ragi (*Eleusine coracana* Gaertn.). Ann. Appl. Biol. 9: 184–186. 1922.
4. McRAE, W. Economic Botany, III. Mycology. Ann. Rep. Board Sci. Advice, India, 1922–1923, pp. 21–22. 1929.
5. MOORE, M. B. A method of inoculating wheat and barley with loose smut. Phytopath. 26: 397–400. 1936.
6. MUNDKUR, B. B. Karnal bunt, an air-borne disease. Curr. Sci. 12: 230–231. 1943.
7. ——————, and M. J. THIRUMALACHAR. Revisions of and additions to Indian fungi. I. Mycol. Pap. Imperial Mycol. Inst. No. 16. 1946.
8. THIRUMALACHAR, M. J. A method of germinating and staining teleutospores. Jour. Indian Bot. Soc. 19: 71–75. 1940.

FUNGI PATHOGENIC TO BLUEBERRIES IN THE EASTERN UNITED STATES

J. B. DEMAREE AND MARGUERITE S. WILCOX[1,2]

Several species of blueberry (*Vaccinium*) are indigenous in the United States. The lowbush species often form dense clumps, or grow thickly over wide areas on uncultivated lands where there is little competition by larger shrubs or trees. In such situations, pathogenic fungi find conditions favorable for rapid spread. Severe leaf infections resulting in extensive preharvest defoliation sometimes occur in wild growth of the lowbush species *Vaccinium angustifolium* Ait.[3] and *V. myrtilloides* Michx. in Maine, and *V. vacillans* Torrey and *V. pallidum* Ait. in more southern regions. The highbush swamp blueberry, *V. australe* Small, grows wild in the Atlantic Seaboard States, and the rabbiteye species, *V. ashei* Reed, in southern Alabama, southern Georgia, and northern Florida. These two highbush species, while sometimes found in dense and extensive stands, occur mostly in clumps of a few bushes, or singly and scattered. Consequently the opportunity for dissemination of spores of pathogenic fungi from one bush to another, or to groups of bushes, is less favorable than if the plants were massed. Then, too, individual seedlings of the species may have a genetic resistance to pathogenic fungi.

The highbush swamp blueberry is now grown commercially in Massachusetts, Michigan, North Carolina, New Jersey, and New York. The rabbiteye form is cultivated in Alabama, Florida, Georgia, Louisiana, and Mississippi.

When blueberry varieties are grown under cultivation in solid stands of about 1,400 bushes per acre, the opportunity for spread of fungus spores from one bush to another is obviously much greater than in the case of scattered wild bushes.

With few exceptions all known parasitic fungi of the cultivated blueberry are restricted to *Vaccinium* and presumably are indigenous. Doubtless they were present in wild blueberries long before cultivated fields were established. Usually blueberries are cultivated on soils and in localities where the wild forms thrived, and it is not unusual for cultivated blueberry fields to be surrounded by forests, swamps, or cut-over lands where wild blueberry bushes form a portion of the flora, a condition very favorable for the spread of diseases from the wild to cultivated fields. A good illustration

of this type of spread of blueberry fungi has been reported by the writers (7) in the case of the blueberry cane canker caused by *Physalospora corticis* Demaree and Wilcox. This fungus is not known to be present in New Jersey, a section where the blueberry is more extensively cultivated and propagated than elsewhere. When New Jersey-grown nursery bushes are planted in North Carolina they often become infected with the canker fungus as the result of the spread of the disease from infected wild bushes near the fields. This canker fungus has been found in wild bushes growing not only in close proximity to cultivated blueberry fields but also in localities remote from blueberries under cultivation.

Lesions caused by most leaf inhabiting pathogens of blueberry are similar in size, color, and markings. As a consequence field determinations are often exceedingly difficult to make; and therefore it frequently is necessary to postpone definite identification until examination can be made by laboratory techniques.

This paper is an attempt to bring together in one publication the present knowledge concerning fungi pathogenic to blueberries, especially cultivated blueberries grown in the Atlantic Seaboard and Gulf States. Included is information about the occurrence and distribution of common and widespread forms of blueberry pathogens, some reported but little known, others reported but rarely seen, and a few heretofore not reported. With a few exceptions, the fungi herein discussed include only those that have been collected or observed by the writers. The list may not be complete, especially for forms inhabiting wild species and for those of the cultivated rabbiteye blueberry (*Vaccinium ashei*). We have a few undetermined collections of the latter.

SEPTORIA ALBOPUNCTATA CKE.

This blueberry pathogen was described by Cooke (5) in 1883 from leaves of *Vaccinium arboreum* Marsh collected in Florida and North Carolina. No later collection of the fungus or mention in literature was known to the writers previous to their collection of it from greenhouse plants at the Plant Industry Station, Beltsville, Md., in 1939. This occurrence was on plants formerly used by the late F. V. Coville in his breeding experiments at Washington, D. C. The source of the fungus found on those plants is unknown. Dr. Coville collected plants from various regions and evidently brought the fungus in on some material. The fungus has not been found in wild or cultivated plants in the vicinity of Washington, D. C. The writers have since collected the fungus on *V. australe* in U. S. Department of Agriculture experimental plots near Atkinson and Ivanhoe, N. C., and Brunswick, Ga.; also on *V. ashei* at the Coastal Plain Experiment Station, Tifton, Ga., and in commercial plantings near Crestview, Fla.

Cooke described the leaf lesions resulting from infection by *Septoria albopunctata* as small, circular, white within, and with a purple border. Usually the spots have the appearance thus described, but sometimes the

centers are tan to russet with a peripheral zone of brown, or the spot may be entirely brown.

Cooke made no mention of the attack on young shoots, though this sometimes occurs on the current year's growth. In general, lesions on shoots resemble those formed on leaves (Fig. 1, A, D), except that the shoot lesions are larger. Single lesions may be 5 to 6 mm. in diameter, circular to irregular, tan to gray, slightly sunken, and surrounded by a zone of reddish brown. A single pycnidium is usually present within the confines of the lesion.
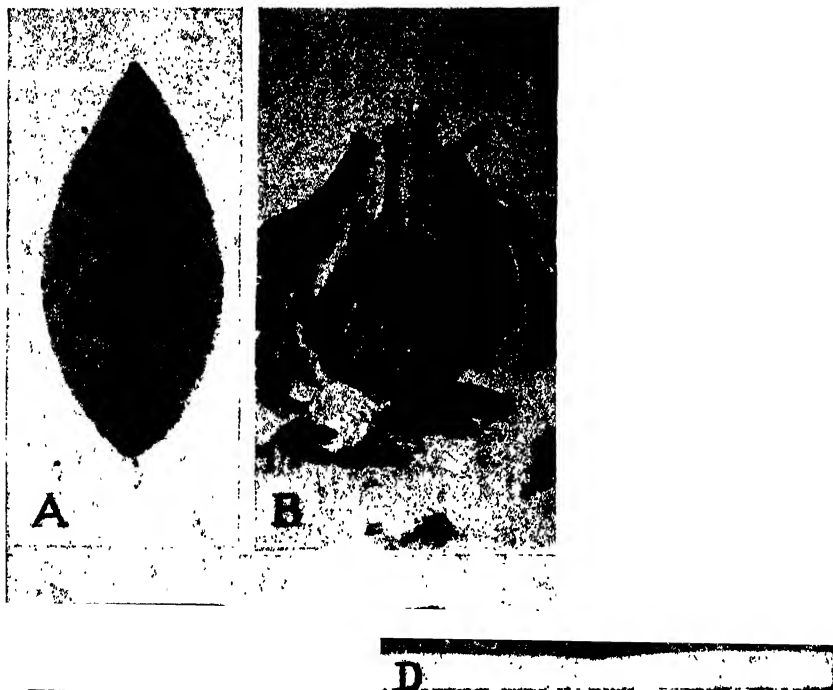
FIG. 1. *Septoria albopunctata* Cke. A. Blueberry leaf naturally infected with *S. albopunctata.* × 1. B. Cross section of a pycnidium. × 335. C. Pycnospores. × 365. D. Natural infection on blueberry shoot. × 1.

The fruiting body of *Septoria albopunctata* is a well developed pycnidium (Fig. 1, B) on the upper leaf surface. Upon maturity the ostiole breaks through the epidermis. On the leaves there is usually one pycnidium in a spot, but occasionally as many as four or five. The pycnidia are ovoid and average about 118 μ high and 90 μ wide. The walls are 4 to 6 μ thick and composed of 2 to 4 layers of cells.

The pycnospores are hyaline, straight or curved, 5- to 11-septate, filiform, obclavate to spindle-shaped, often with a long, attenuated apical segment (Fig. 1, C). When formed in host tissue they vary from 42 to 96 μ long and 3.0 to 4.8 μ wide, averaging about 70 μ by 3.6 μ. The sporophores average 12.0 μ long. When the fungus was grown on cornmeal agar the pycnospores produced were about twice the length of those measured from host tissues.

of a perfect stage, but none ever developed that could be associated with the *Dothichiza*.

The fungus grew slowly on agar media and became delimited before covering the slant in tube cultures. No type of fructification ever appeared in cultures, even though a variety of media was used and temperatures were varied. When autoclaved blueberry leaves were used as the substratum, sclerotial bodies formed within the tissues, but no pycnidia developed.

This *Dothichiza* leaf disease has been seen in almost all blueberry fields visited in North Carolina. Apparently all varieties of the highbush swamp blueberry are susceptible to infection. There is, however, some difference in degree of susceptibility. Cabot, Dixi, Pioneer, and Rancocas are the most susceptible varieties. Adams, Concord, Jersey, and Weymouth are moderately susceptible, and Grover, Harding, June, and Sam have considerable resistance.

Dothichiza caroliniana sp. nov.

Pycnidia in maculis primum 1–4 mm. diam., demum majoribus, brunneis vel griseis plerumque epiphylla, 1–5 in quaque macula, subepidermalia, erumpentia, atra, conica vel irregularia, 80–130 μ alta, 48–95 μ lata, non-ostiolata, pariete 12 μ crasso; pycnosporae hyalinae, continuae, $7.0 \times 2.2 \mu$, utrinque obtusae, e sporophoris brevibus orientes. Hab. in foliis Vaccinii australis, North Carolina.

Spots at first 1 to 4 mm. in diameter, variable in shape, dark brown, later light brown or gray in center, often developing after midsummer a secondary necrotic area around the original spot (Fig. 2, A). Pycnidia mostly epiphyllous, usually 1 to 2 but sometimes 4 or 5 on each spot, subepidermal at first, later erumpent, slightly imbedded in host tissues, black, conical to irregular, 80–130 μ high by 48–95 μ wide (Fig. 2, B). Wall thick, about 12 μ, non-ostiolate. Pycnospores hyaline, continuous, 7.0 by 2.2 μ, obtuse at each end (Fig. 2, C), growing from base of pycnidium on short sporophores. Habitat in living leaves of *Vaccinium australe* Small in North Carolina. Type specimen, collected by J. B. Demaree at Magnolia, N. C., July 22, 1940, deposited in Mycological Collections, Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Md., under No. 71489.

PHYLLOSTICTINA VACCINII SP. NOV.

A foliage disease caused by the fungus herein described as *Phyllostictina vaccinii* was first observed by the writers on cultivated bushes of *Vaccinium australe* near Beltsville, Md., in 1939. Later collections have been made on other *Vaccinium* species, both cultivated and wild, in Georgia, North Carolina, Maryland, and Mississippi. In 1942 Dr. Donald Folsom, of the Maine Agricultural Experiment Station, Orono, Me., sent the writers several cultures of fungi isolated from leaves of wild blueberries in Maine. When grown on artificial media, one of those isolates appeared to be identical with

Phyllostictina from the southern blueberry. This fungus probably is widely distributed in the eastern and southern United States, and, although unimportant as a leaf pathogen, it is potentially important as the cause of a fruit rot, since it regularly attacks fruits of one variety of *Vaccinium ashei*.

Phyllostictina vaccinii is primarily a parasite on blueberry leaves and causes small circular spots, grayish in the center and surrounded by a zone of brown host tissues (Fig. 3, A). Usually one pycnidium forms on a spot, occasionally as many as five or six. Affected bushes sometimes are partially defoliated near the end of the growing season.

Both leaves and fruits of the Black Giant, a variety of the rabbiteye blueberry (*Vaccinium ashei*), are regularly attacked. Mr. Otis Woodard estimated that 50 per cent of the berries on the Black Giant variety were infected at the Georgia Coastal Plain Experiment Station in 1942 (correspondence). On the fruits the disease appears during the preripening period as a hard, dry rot, localized in spots 5 to 6 mm. in diameter, grayish and sunken, with numerous black pycnidia in the central region (Fig. 3, B). The spots are conspicuous on the black background of ripe berries and the affected ones must be sorted out before marketing.

The fruiting bodies of the *Phyllostictina* conform to the usual concept of a pycnidium. Their size varies considerably, depending upon the substratum. On the leaves of either the rabbiteye or the highbush blueberry they average about 75 μ high and 68 μ wide, but on the fruit of the Black Giant they are much larger, averaging about 188 μ high and 165 μ wide.

The pycnospores are globose to ovoid, hyaline, granular, and average about 7.6 by 7.0 μ when formed on either leaves or fruit. Young spores (Fig. 3, E) usually possess an inconspicuous long, narrow, hyaline appendage, varying in length from 32 to 96 μ. The appendages were most clearly seen and the length easily determined when the pycnospores were first mounted in water, between a slide and cover glass; the water was then allowed to evaporate, whereupon the appendages showed distinctly. They are narrow, continuous, and frequently curved or bent near the free end.

Stained sections of the pycnidia in different stages of their development showed that the locular contents of the young fruiting bodies were composed of thin-walled pseudoparenchyma tissue (Fig. 3, C). The cells in the central region were smaller than those near the pycnidial wall. Histolysis took place progressively from the center of the pycnidium toward the periphery (Fig. 3, D). This process continued until all the original locule contents disappeared and the space was then packed with the globose, hyaline spores. Apparently no sporophores were formed during this stage of spore development, or, if formed, they became evanescent during an early period of their development. After this crop of pycnospores was discharged a second light crop of spores was formed on sporophores originating from the inner surface of the pycnidium. The sporophores were as long as the width of the spores.

This blueberry *Phyllostictina* grew well on nutrient agars and covered tube slants in a few days when held at room temperature. The agar turned black on the surface and to the depth of the penetrating mycelium. There were some mouse-colored aerial hyphae with patches of white. Prominent
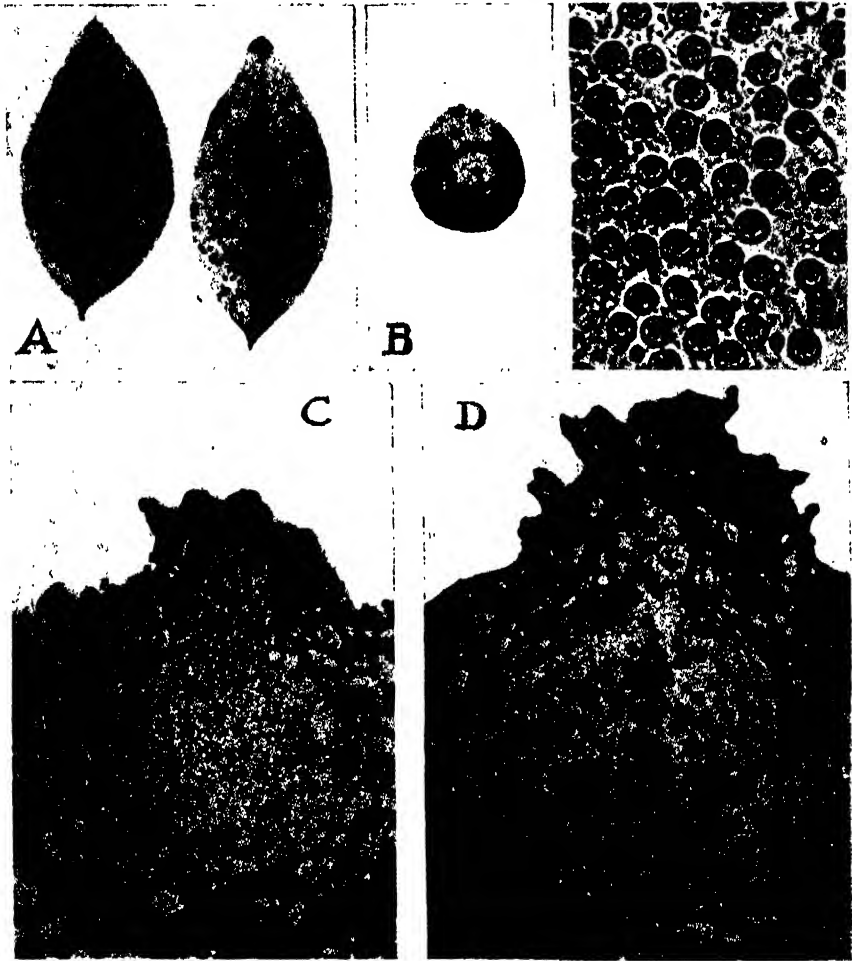
FIG. 3. *Phyllostictina vaccinii* sp. nov. A. Blueberry leaves with spots caused by *P. vaccinii.* ×1. B. Fruit of an infected rabbiteye blueberry. ×2. C. Cross section of a young pycnidium before formation of pycnospores. ×335. D. Cross section of a more mature pycnidium in process of forming pycnospores, peripheral cells of pycnidium contents still intact. ×335. E. Globose pycnospores of the fungus, some showing their delicate appendages. ×490.

stromatic bodies bearing what appeared superficially to be fruiting structures commonly formed on cornmeal agar. These structures varied in shape from spherical to tubular and we have frequently examined them with the expectation of finding them filled with pycnospores, but in all cases they were sterile. Occasionally when this fungus was grown on corn-

meal agar it formed a few slimy masses of spherical hyaline conidia on the surface of the agar, independent of any fruiting structure. The conidia were of the same size as and otherwise appeared identical with pycnospores formed in host tissues.

There is undoubtedly merit in the present tendency to break down the large and unwieldy *Phyllosticta-Phoma* form-genus when the characters of forms critically studied permit separation and assignment to some other genus.

Sydow's policy (19) of placing in the genus *Phyllostictina* those forms having fruiting bodies similar to *Phyllosticta* and filled at first with pseudo-parenchymatous tissue, which later is transformed by histolysis into spores, seems to be sound. Von Hoehnel's modification (10) admits to this genus the forms having pycnidia with pseudoparenchymatous tissue subject to histolysis and producing spores having evanescent conidiophores. Shear (18) admits the presence of sporophores in a pycnidium filled with paren-chymatous tissue subject to histolysis, but prefers to restrict the name *Phyllostictina* to the imperfect stage of *Guignardia*.

During the preparation of this paper some other fungi listed in litera-ture and possibly identical with this blueberry *Phyllostictina* were ex-amined when specimens were available, and in other cases the literature was reviewed. The following described fungi were considered: *Phoma leptidea* (Fr.) Sacc. (17) on *Vaccinium uliginosum* L., described from dead fallen leaves and having straight or curved spores 8 by 2μ; *Phoma cymbi-spora* (Berk. and Curt.) Sacc. (16), growing on branches of *V. uliginosum* and having tinted boat-shaped spores 15μ long; *Phyllosticta amicta* Ell. and Ev.[4] (9) on leaves of *Arctostaphylos sp.*, producing straw-colored spores measuring 10 by 7μ; *Phyllosticta vaccinii* Earle (8) on *V. arboreum* reported as having spores 12 by 6μ; *Phyllosticta cyanococci* Dearn. and House (6) on *V. corymbosum* L., reported as having spores 3 to 4 by 1.25μ; and *Phyllosticta sparsa* Bonar (3) on leaves of *V. ovatum*, reported as having pycnidia reaching 150μ in diameter with globular, hyaline, granular spores 12 by 9μ, produced on simple short conidiophores up to the diameter of the spore in length.

Bonar's *Phyllosticta sparsa* resembles the eastern blueberry fungus more closely than any other fungus compared. It has pycnidia and spores larger than those of our fungus, but probably not sufficiently so to be significant. Spores of *Phyllosticta sparsa* were reported to measure 12 by 9μ, compared to 7.6 by 7.0μ for *Phyllostictina vaccinii*. In addition to differences in spore size, Bonar notes the presence of conidiophores and does not mention presence of spore appendages, a common character for *Phyllostictina*. Until the two forms can be compared more critically it appears best for the present to consider them as separate entities.

[4] A specimen purporting to be *P. amicta* was examined and found to be quite dif-ferent from the blueberry *Phyllosticta*.

Phyllostictina vaccinii sp. nov.

Maculae in foliis plerumque 1–3 mm. diam., badiae usque griseae margine purpureo, in fructibus circulares, 6–8 mm. in diam., griseae, planae vel depressae, durae; pycnidia in foliis parca, 1–6 in quaque macula, subglobosa usque globose, epiphylla, subepidermalia, ostiolata, circa 75 μ alta, 68 μ lata, pariete crasso subcarbonaceo, in fructibus numerosa, 188 μ alta, 165 μ lata, cavitas pycnidica primum e contextu pseudoparenchymatico hyalino, dein pycnosporis ex histolysi oriundis repleta; pycnosporae subglobosae vel globosae, hyalinae, granulosae, continuae, circa 7.6 × 7.0 μ, appendicibus usque 96 μ longis, hyalinis, tenuibus praedita; conidiophori solum post liberationem conidiorum histolyticorum praesentes. Hab. in foliis Vaccinii australis, V. atrococci, et V. pallidi × atrococci, et in foliis fructibusque V. ashei, Georgia, Mississippi, Maryland, et North Carolina.

Spots on leaves mostly 1 to 3 mm. in diameter, russet to gray surrounded with purple margin. Spots on fruits circular, 6 to 8 mm. across, gray, flat or depressed, hard, with numerous black pycnidia. Pycnidia on leaves few, usually 1 to 6, oval to globose, averaging 75 μ high and 68 μ wide, on upper surface of leaf, subepidermal, ostiolate; wall thick, subcarbonaceous. On fruit, pycnidia larger, averaging 188 μ high and 165 μ wide. Pycnidial cavity first filled with hyaline pseudoparenchyma tissue, transforming by histolysis into ovoid to globose, hyaline, granular, nonseptate pycnospores averaging 7.6 by 7.0 μ and having long, hyaline, delicate appendages up to 96 μ long. Conidiophores formed only after discharge of histolysis-formed conidia.

On leaves of *Vaccinium ashei* Reade, Ellisville, Miss., Tifton, Ga., Willard, N. C., and Beltsville, Md.; *V. australe* Small, Beltsville and Salisbury, Md., Magnolia and Willard, N. C.; *V. atrococcum* (A. Gray) Heller, Buchanan, Ga.; *V. pallidum* × *atrococcum* segregate, Beltsville, Md.; and on fruits of Black Giant (*V. ashei*), Tifton, Ga. Type specimen collected by J. B. Demaree on *V. ashei*, Willard, N. C., July 30, 1942, deposited in Mycological Collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Md., under No. 71490.

GLOEOCERCOSPORA INCONSPICUA SP. NOV.

This fungus causes spotting of blueberry leaves and sometimes is the direct cause of premature defoliation. Collections of spotted leaves have been taken in cultivated fields of the highbush blueberry, *Vaccinium australe*, near Ivanhoe, Atkinson, and Magnolia, N. C.; on wild *V. australe* near Beltsville, Md.; and on the rabbiteye blueberry, *V. ashei*, near Ivanhoe, N. C.

The brownish lesions are circular to angular, and do not differ greatly in appearance from spots caused by *Dothichiza* before the secondary spread of the latter occurs (Fig. 4). For this reason the foliar effects caused by the two fungi are sometimes difficult to distinguish in the field.

The sporodochia of *Glococercospora* are formed more frequently on the upper than on the lower epidermis. Although there may be a dozen or more sporodochia on a spot they are usually extremely inconspicuous if the leaf surface is dry, and they often are entirely overlooked. When dry the sporodochia appear as thin, flat discs, slightly raised, and about the same color as the host tissue. When a thin film of water is spread over leaf lesions bearing the inconspicuous structures, these bodies immediately enlarge, more in height than radially, and then appear as clear, glistening, soft, spherical to conical globules. When the film of water evaporates, the

FIG. 4.   Spots on blueberry leaves caused by *Glococercospora inconspicua* sp. nov.

gelatinous masses revert to the original flat disc form. This behavior is useful in identification of the disease in the field.

A satisfactory method of preparing material for microscopical study of the sporodochia has not been devised. Only a small portion of the structures remained after material was dehydrated, imbedded, sectioned, and stained in preparation for permanent mounts. Better success was obtained when hand sections were made. This method demonstrated that the sporodochia were under the cuticle or slightly imbedded in the epidermis. They measured from 40 to 90 μ wide and 36 to 60 μ high. Some were constricted at the base, as if possessing a short stipe, and widened out in a broader top. The body of the sporodochium was an undifferentiated opaque mass with some intermingling of hyphal strands. The hyphae were interpreted as sporophores remaining intact. A few conidia were usually clinging to or partly imbedded in the sporodochial cushions, and some have been seen attached to hyphae projecting slightly above the otherwise formless masses.

The conidia are hyaline, usually curved, septate, and about 35 μ long and 3 μ wide (Fig. 5). When germinating, one or more of the segments may form germ tubes.

Growth was weak and submerged on cornmeal agar and no reproductive bodies of any kind were formed. The hyphae were fine, hyaline, and septate.

A study of the early development of the sporodochia has not been made, and until that can be done one can only speculate as to the origin and morphology of the structures. The writers hold the theory that at first there is formed a palisade of simple upright conidiophores bearing conidia apically. After the conidiophores push through the cuticle and become wet by dew or rain they transform into the undifferentiated jellylike mass. The conidia are probably dispersed soon after being formed.

FIG. 5. *Gloeocercospora inconspicua* sp. nov. Conidia drawn by aid of a camera lucida. × 1100.

This blueberry fungus is thought to be referable to *Gloeocercospora*, a genus founded by Bain and Edgerton (1) in 1943 in connection with their description of the monotypic *G. sorghi*, pathogenic on *Sorghum halepense*. The two fungi are similar in several essential characters. *G. inconspicua* differs from the sorghum fungus in that its sporodochia are smaller and less conspicuous and are not confined to the stomata; the conidiophores are more completely dissolved; conidia are not formed in great abundance; growth is weak and the fungus does not sporulate in artificial culture media; and does not form sclerotiumlike bodies in artificial media or in infected host tissues.

Gloeocercospora inconspicua sp. nov.

Sporodochia in maculis fuligineis vel brunneis, interdum cinerascentibus, circularibus, 2–5 mm. in diam., epiphylla, sicca inconspicua, madida in forma globulorum gelatinosorum evidentia, subcuticularia, 45–96 μ lata, 60–85 μ alta; conidia filiformia, hyalina, curvata, septata, 35 × 3 μ; conidiophora hyalina, simplicia, madida gelatinosa. Hab. in foliis Vaccinii australis parasitica, North Carolina, Georgia, et Maryland.

Spots on leaves sooty to brown, sometimes grayish, circular to angular,

2 to 5 mm. in diameter, showing on both leaf surfaces. Sporodochia epi-
phyllous, inconspicuous when dry, appearing as gelatinous globules when
wet, subcuticular, 45–96 μ wide and 60–85 μ high. Conidia filiform, hyaline,
curved, septate, 35 by 3 μ. Conidiophores hyaline, simple, becoming gelatin-
ous when wet. Parasitic in leaves of *Vaccinium australe* in North Carolina,
Georgia, and Maryland.

Type specimen collected by J. B. Demaree at Ivanhoe, N. C., on *Vaccin-
ium australe*, August 26, 1935, and deposited in Mycological Collections,
Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S.
Department of Agriculture, Beltsville, Maryland, under No. 71491.

MONILINIA VACCINII-CORYMBOSI (READE) HONEY

On April 8, 1940, the senior author, in company with N. H. Loomis of
the U. S. Horticultural Field Station at Meridian, Miss., visited a small
cultivated planting of the rabbiteye blueberry (*Vaccinium ashei*) near
Ellisville, Miss. At that time new shoots were 2 to 6 inches long and the
bushes were blooming. There was an extensive blighting of the new shoots
and blossoms of many bushes, suggestive of a severe attack of the mummy-
berry disease, often destructive in blueberry plantings in New Jersey,
Massachusetts, New York, Michigan, and Maryland, caused by the fungus
Monilinia vaccinii-corymbosi (Reade) Honey. This Mississippi blueberry
planting is a nonclonic assemblage of seedlings of the rabbiteye species;
consequently there was considerable variation in susceptibility of individual
bushes. While three-fourths or more of the shoots of some bushes were
blighted, other bushes were moderately blighted, and still others had only
a few, or no, blighted shoots or blossoms.

A search was made under the bushes for mummied berries and apothecia,
but none were found. Later examination of blighted blossoms and shoots
collected from the bushes April 8 did show the presence of monilioid
conidia, 19–29 × 14–26 μ, connected by disjunctors.

Mr. Loomis visited this blueberry planting again during the summer,
when he collected mummied berries of the current year's crop. The berries
contained pseudosclerotium structures typical for the genus *Monilinia*.

Mummied berries and blighted shoots and blossoms collected from the
Mississippi rabbiteye blueberry planting were sent to Edwin E. Honey,[5] an
authority on the North American species of the genus *Monilinia*. He re-
plied that the fungus was undoubtedly a *Monilinia*, but that, in the absence
of the perfect stage, determination of the species could not be made. He
further said, "As far as I know, this is the first report of a *Monilinia* on
Vaccinium virgatum (*V. ashei*)."

Dr. Honey visited the Ellisville, Miss., blueberry planting the following
April (1941). Fortunately, he timed his visit to coincide with a period when
both apothecia and conidia were present. He concluded that the fungus

was identical with *Sclerotinia vaccinii-corymbosi* Reade, which he recognizes under the name of *Monilinia vaccinii-corymbosi* (Reade) Honey.

The mummy-berry disease occurs sporadically in the northeastern United States, and sometimes causes considerable damage to the fruit of the high-bush swamp blueberry (*Vaccinium australe*). Where the fungus is present, an epidemic of the disease is contingent upon spring weather conditions favorable to the development of apothecia and dissemination of ascospores.

For the past 10 years the writers have been on the lookout for this *Monilinia* in the thriving blueberry-growing section of North Carolina, but never observed it until the spring of 1945, when a few mummied berries were seen at harvest time in one planting near Ivanhoe, N. C. This observation extends the known range of *Monilinia vaccinii-corymbosi* (Reade) Honey on *Vaccinium australe* much farther south than heretofore reported.

There is no indication except the Ellisville, Miss., observation whereby the potential seriousness of the disease in the South can be predicted.

BLUEBERRY PATHOGENS WIDELY DISTRIBUTED AND WELL KNOWN

The few blueberry pathogens critically examined and reported in the preceding sections are by no means the only ones known.

Microsphaera alni

The powdery mildew, *Microsphaera alni* DC. ex Wint., is the most wide-spread of all blueberry pathogens encountered. It is found at some time every summer in all blueberry fields from Maine to Florida; it causes from slight to moderate damage, and occasionally serious damage. Markin reported (11) that mildew is of common occurrence on *Vaccinium pennsylvanicum* var. *nigrum* (*V. brittonii* Porter & Beckn.) in Maine and is the cause of much early defoliation. She also reported observation of the fungus on *V. corymbosum* (*V. australe* Small), *V. canadense* (*V. myrtilloides* Michx.), and on *V. pennsylvanicum* (*V. lamarckii* Camp). The first-named species was reported to have shown very striking individual differences in susceptibility.

Bergman's (2) studies on the variation in the amount of mildew on 10 cultivated varieties in Massachusetts indicate severity of attack as follows: Pioneer, most susceptible, with Cabot and Wareham as close competitors; Concord, Jersey, and Rubel, intermediate in susceptibility; Stanley, Rancocas, Harding, and Katherine, highly resistant. From a genetic standpoint it is interesting to note that Pioneer, the most susceptible variety, and Katherine, the most resistant, are of the same parentage. Meckstroth's[6] observations in the cultivated fields in North Carolina indicate similar resistance in varieties. In his opinion Pioneer is the most susceptible variety, closely followed by Cabot. Jersey, Sam, and Stanley were moderately mildewed; Adams, Concord, and Rubel had high resistance; and a trace of

---

[6] Data taken from observations made by G. A. Meckstroth, Associate Pathologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

the fungus was observed on Dixi, Harding, Grover, June, Rancocas, and Weymouth.

The powdery-mildew fungus does not attack all varieties in the same manner. On some the fungus is principally confined to the upper leaf surface, forming a white compact layer of hyphae, either localized or covering the entire surface. On other varieties the hyphae are sparse, inconspicuous, and confined to the lower leaf surface, causing superficial reddish areas where the creeping hyphae form attachments. On the upper surface of the leaves such areas are faintly yellowish spots.

Pucciniastrum myrtilli

The heteroecious rust fungus, *Pucciniastrum myrtilli* (Schum.) Arth., is about as widespread some years as the powdery-mildew fungus and likewise occurs on all *Vaccinium* species. It is most commonly found within the range of its alternate aecial host, *Tsuga sp.* This rust, however, is not confined to the regions where the hemlock is indigenous. The heaviest infection of rust on cultivated blueberry leaves has been observed by the writers in the Coastal Plain sections of North Carolina, Georgia, and Alabama—sections at least 200 miles remote from native hemlock growth. The most southern collections of *P. myrtilli* (uredia) were taken near Brunswick, Ga., on *Vaccinium australe*, May 20, 1939; Crestview, Fla., on *V. ashei*, September 25, 1939; and Boston, Ga., on *V. ashei* June 12, 1945. Abundance of the fungus in these southern sections is unusual. During the past 11 years it has been either rare or absent in North Carolina blueberry fields, except during 1938, 1939, and 1946, when the rust disease developed in epiphytotic form over a wide area in North Carolina, Florida, and Alabama and caused some premature defoliation near the end of the growing season. In 1945 the disease was so severe at Boston, Ga., on *V. ashei* that the early-formed leaves were falling and new foliage was developing prior to the middle of June. This presumably was a local outbreak. The epiphytotic outbreak in 1946 was about as severe- in some blueberry fields in North Carolina as in 1938 and 1939. In 1946 this rust was observed for the first time in a blueberry field on the Eastern Shore of Maryland. An abundant infection occurred which caused considerable premature leaf fall in October. Only a light infection was observed in New Jersey that year.

The sporadic outbreaks of the rust fungus in regions remote from hemlock may be explained by assuming that there is regular overwinter survival of the uredia on southern wild species of *Vaccinium* with evergreen leaves. It is also possible that some uredia will survive in plantings of deciduous blueberries during mild winters when some leaves remain green all winter. If that is true, then the fungus could increase to an epiphytotic stage over a period of two or three years of recurrent mild winters. There is also a possibility that the occasional epiphytotics of *Pucciniastrum myrtilli* in North Carolina, south Georgia, and south Alabama originate

from spores brought in by upper air currents from some remote region where the wild hemlock grows.

Markin (11) reports that *Pucciniastrum myrtilli* is general in distribution in Maine and occurs as far as ⅛-mile from hemlock trees. She has observed the rust on *Vaccinium canadense* (*V. myrtilloides* Michx.), *V. corymbosum* (*V. australe* Small), *V. uliginosum* L., and *Gaylussacia baccata* (Waugh.) K. Koch.

According to Meekstroth, cultivated varieties of the highbush blueberry are variable in their susceptibility to leaf rust. He surveyed a large planting in 1939 near Ivanhoe, N. C., composed of several varieties, all similarly exposed to infection. This examination showed that the ·Harding and Grover varieties were very heavily infected; Adams, Concord, Jersey, and Rubel, heavily infected; Stanley and Sam, moderately infected; Cabot, slightly infected; but no rust was found on Dixi, June, Pioneer, Rancocas, and Weymouth. In this same field in 1946, when such varieties as Grover, Sam, and Adams of the highbush blueberry were very heavily infected and were completely defoliated by early November, the varieties Owens, Myers, and Black Giant of the rabbiteye species were only slightly infected.

Although leaf rust has been reported on species of *Vaccinium* from the far western States, aecia of *Pucciniastrum myrtilli* have not been found on western hemlock species and it is suspected that the leaf rust on western *Vaccinium* either is not *P. myrtilli*, or, if it is, has some unknown alternate host.

Pucciniastrum goeppertianum

Pucciniastrum goeppertianum (Kühn) Kleb. is parasitic on certain *Ericaceae* and on species of *Abies*. It causes a disease known as witches'-broom on blueberries, which has been reported from Maine south to Pennsylvania and west to California and Washington. This disease has not been seen south of New England in cultivated blueberry fields in the East. Markin (12) says that the disease is frequent on wild blueberry plants growing within several hundred yards from woodlands and becomes serious in small areas partially surrounded by woods. In Maine the disease has been observed, according to Markin, on *Vaccinium caespitosum* Michx., *V. canadense* (*V. myrtilloides* Michx.), *V. corymbosum* (*V. australe* Small), *V. pennsylvanicum* (*V. lamarckii* Camp), *V. uliginosum* L., and *V. vitis-idaea* L. Markin states that the cultivated blueberry varieties Adams, Cabot, Harding, Pioneer, Rubel, and Sam are more susceptible to witches'-broom than are native Maine species. The varieties Katherine and Rancocas showed evidence of being resistant.

Physalospora corticus

The fungus *Physalospora corticus* Demaree & Wilcox (7) causes a cane canker of considerable economic importance in cultivated blueberries in the southeastern United States. The disease is present in most plantings of

the highbush blueberry, *Vaccinium australe*, in North Carolina and on the cultivated rabbiteye blueberry (*V. ashei*) in Alabama, Florida, and Mississippi. It has also been found on wild *V. australe* in North Carolina and in wild growths of *V. ashei* in Florida. Varieties show a decided difference in their ability to resist attacks of *P. corticus*. Of the highbush varieties, Cabot is the most susceptible and is rapidly being discarded in the South for that reason. Other observed varieties in descending order of their susceptibility are: Pioneer, Concord, June, Stanley, Jersey, Scammell, Rancocas, and Rubel. Named varieties of the rabbiteye blueberry examined for resistance are Black Giant, Hagood, Locke, Walker, Ethel, Myers, Owens, Ruby, Clara, and Scott. Cankers were found only on Locke.

BLUEBERRY PATHOGENS OF MINOR ECONOMIC IMPORTANCE OR RARELY SEEN

Twig blight, a minor disease of cultivated blueberries, is sometimes present in Massachusetts and New Jersey. This type of twig necrosis was first thought to be a form of winter injury. Later a *Phomopsis* was isolated from blighted twigs and produced the disease when the fungus was artificially inoculated in blueberry plants. The fungus is thought to enter through leaves or through the succulent shoot tips and to progress slowly downward into the scaffold branches, where it becomes perennial. It later penetrates all parts of the plant, finally causing death of the bush. The extreme symptoms are seldom obtained, since weak bushes are taken out before there is much deterioration. The signs usually seen are dead tips, from a few inches in length to 12 or 18 inches. Growers confine the incidence of the disease to a low level by pruning out the diseased shoots, cutting well below the dead portion, and burning the pruned material. Miss Wilcox (20) showed that the *Phomopsis* associated with twig blight is identical with *P. vaccinii* Shear, Stevens, and Bain, a cranberry fruit-rotting fungus. She (21) later discovered that the *Phomopsis* produced a perfect stage in culture indistinguishable from *Diaporthe vaccinii* Shear.

Brown (4) reported upon a blueberry stem and crown gall occurring on cultivated varieties in Massachusetts, Michigan, New Jersey, and Oregon, and attributed the cause to a species of *Phomopsis*. This *Phomopsis* is unlike the one causing twig blight.

Botrytis sp. is sometimes the cause of considerable loss of cultivated blueberries. The fungus attacks the blossoms, fruits, and succulent leaves during prolonged periods of foggy weather. The disease more commonly occurs in Oregon, occasionally in New England, and rarely in New Jersey.

Exobasidium vaccinii (Fckl.) Wor. has been collected on *Vaccinium* species from Maine southward to Maryland and westward to Texas and Washington. It causes a blueberry disease of minor importance but of interest because of the hypertrophy and brilliant red color of infected blossoms, fruits, and leaves.

In 1939 H. F. Bergman visited a blueberry planting on Long Island, N. Y., where severe defoliation was taking place. Fruiting bodies of a

Gloeosporium were abundant on the leaves. Specimens of diseased leaves were submitted to the writers and the fungus was determined as the imperfect stage of *Glomerella cingulata* (Stonem.) Spauld & Schrenk, the apple bitter-rot fungus. The fungus was isolated and proved to be virulent when inoculated on young leaves, succulent shoots, and green berries of potted blueberry plants (*Vaccinium australe*) in the greenhouse.

A few additional fungi pathogenic in blueberries have been collected by the writers, but they have not been critically studied. They include the following: *Ramularia effusa* Pk. on *Vaccinium vacillans* Torrey, Beltsville, Md., and on *V. lamarckii* Camp, Cherryville, Maine; *Rhytisma vaccinii* (Schw.) Fr. on *V. atrococcum* Heller, Beltsville, Md.; and *Cercosporella sp.* on *V. australe* Small, near Weymouth and New Gretna, N. J. Both collections of the last named fungus were made in 1940 and it has not been seen since. The fungus caused circular to irregular lesions 2 to 5 mm. in diameter, showing on both surfaces of green leaves. On the lower surface the spots had a slightly frosty appearance owing to a thin layer of hyaline conidia that were about $40\,\mu$ long and $3\,\mu$ wide, 1- to 3-septate, and very much curved.

Several other fungi have been reported as occurring on blueberries. Wilcox (22) listed the following: *Helminthosporium inaequale* Shear, *Melanospora destruens* Shear, *Pestalozzia guepini vaccinii* Shear, *Sphaeropsis malorum* Pk., *Dothiorella ribis* Grov. and Dugg., and *Alternaria sp.*

NAMES OF DOUBTFUL VALIDITY

Septoria difformis Cke. and Pk. was described from living leaves of *Vaccinium pennsylvanicum* collected from Lake Pleasant, N. Y., in 1875. The fungus, according to the authors (14), produced linear, straight or curved, hyaline spores in profusion, about $15\,\mu$ long and "oozing out and covering the spot with a white or glaucous bloom." The writers know of no record of collection of the fungus since. Through the courtesy of Mr. Homer D. House, New York State Botanist, Albany, a portion of the type material of *Septoria difformis* was obtained for examination. The specimen had black pycnidium-like structures on the leaf lesions, but all examined were devoid of spores. Growing between and upon the sterile structure were numerous caespitose brown conidiophores bearing conidia measuring 13–18 by 3–$5\,\mu$. These measurements fall within the range of *Ramularia vaccinii* Pk. (15). The type collection of the latter shows spots of about the same size and character as those in the specimen of *S. difformis*, but none of the black pycnidiumlike bodies are present. However, a specimen determined by H. S. Jackson as *R. vaccinii* Pk. on *Vaccinium pennsylvanicum* (Diamond Lake, Temahami Forest Reserve, Ontario, July 23, 1932) is very similar to the type collection of *S. difformis*, having spots of the same size and appearance, covered on the under side of the leaf by a dense layer of *Ramularia* spores. There were also present in this specimen, particularly around the margin of the spots, sterile black structures resembling pycnidia.

It is not clear why Cooke and Peck described this lowbush blueberry fungus as a *Septoria* when its type specimen indicates it to be a *Ramularia*.

There is one more *Septoria* reported as pathogenic on blueberry whose validity might be questioned. Miss Markin in 1931 reported (12) upon a "brown leaf spot" that was widespread and very destructive to the lowbush blueberry in some areas in the central and southeastern sections of Maine. The following year she reported (13) that the "brown leaf spot (*Septoria sp.*) nowhere caused the complete destruction of foliage that it had in 1930 and 1931." It is not known what Miss Markin was dealing with and referred to as *Septoria sp*. She gave no description of the fungus and, according to Dr. Donald Folsom, no specimens were left with the Maine Agricultural Experiment Station. The senior writer has examined wild blueberry growths in Maine but failed to find any blueberry pathogen that could be classed as a *Septoria*.

SUMMARY

This paper is an attempt to bring together in one publication the known information concerning the occurrence and distribution of fungi parasitic to blueberries (*Vaccinium spp.*) in the United States.

Particular attention has been given to fungi attacking cultivated blueberries grown in the South Atlantic and Gulf States.

Included is a summary of the rather meager literature concerning the more common and widespread forms of blueberry pathogens. Three new pathogenic fungi are described, *Dothichiza caroliniana*, *Phyllostictina vaccinii*, and *Gloeocercospora inconspicua*.

PLANT INDUSTRY STATION,
    BELTSVILLE, MARYLAND.

LITERATURE CITED

1. BAIN, D. C., and C. W. EDGERTON. The zonate leaf spot of sorghum. Phytopath. **33**: 220–226. 1943.
2. BERGMAN, H. F. Observations on powdery mildew on cultivated blueberries in Massachusetts in 1938. Phytopath. **29**: 545–546. 1939.
3. BONAR, LEE. Studies on some California fungi. Mycologia **20**: 292–297. 1938.
4. BROWN, NELLIE A. Blueberry gall produced by the fungus Phomopsis. Phytopath. **28**: 71–73. 1938.
5. COOKE, M. C. New American fungi. Grevillea **12**: 22–83. 1883.
6. DEARNESS, JOHN, and H. D. HOUSE. New or noteworthy species of fungi, IV. Bul. 266, N. Y. State Museum, Dept. of State Botanist. 1924.
7. DEMAREE, J. B., and MARGUERITE S. WILCOX. Blueberry cane canker. Phytopath. **32**: 1068–1075. 1942.
8. EARLE, F. S. New species of fungi from Alabama. Bul. Torrey Bot. Club **24**: 29–32. 1897.
9. ELLIS, J. B., and B. M. EVERHART. New species of fungi from various localities. Proc. Acad. Nat. Sci. Phila. 1895: 413–441. 1896.
10. HOEHNEL, F. VON. Ueber *Phyllostictina Murrayae* Sydow. Ann. Mycol. **18**: 93–95. 1920.
11. MARKIN, FLORENCE L. Notes on blueberry diseases in Maine. U. S. Dept. Agr., Pl. Dis. Reptr. **15**: 11–14. 1931.
12. ——————. Progress of investigations. Maine Agr. Expt. Sta. Bul. 360: 204–205. 1931.
13. ——————. Summary report of progress. Maine Agr. Expt. Sta. Bul. 363: 291–294. 1932.

14. PECK, C. H. Twenty-ninth Report. New York Museum Nat. Hist. 1878.
15. ——————. Thirty-fifth Report. New York Museum Nat. Hist. 1884.
16. SACCARDO, P. A. Sacc. Syll. Fung. **3**: 85. 1884.
17. ——————. Sacc. Syll. Fung. **3**: 111. 1884.
18. SHEAR, C. L. Life history and undescribed genera and species of fungi. Mycologia **15**: 120–131. 1923.
19. SYDOW, H., and E. J. BUTLER. Fungi Indiae orientalis. Ann. Mycol. **14**: 185–186. 1916.
20. WILCOX, MARGUERITE S. Twig blight of blueberry. Phytopath. **29**: 136–142. 1939.
21. ——————. *Diaporthe vaccinii*, the ascigerous stage of *Phomopsis* causing twig blight of blueberry. Phytopath. **30**: 441–443. 1940.
22. ——————. Notes on blueberry fungi. U. S. Dept. Agr., Pl. Dis. Reptr. **20**: 106–107. 1936.

RHIZOSPHAERA KALKHOFFI ASSOCIATED WITH A NEEDLE CAST OF PICEA PUNGENS

ALMA M. WATERMAN [1]

In the spring of 1938 a severe needle cast was observed on more than 200 trees of blue spruce (*Picea pungens* Engelm.) in a planting covering 1.5 acres at Litchfield, Connecticut. The trees were approximately 25 years old and about 20 feet in height. In the original planting the trees were spaced 6 by 6 feet, but subsequently had been thinned by removing alternate rows and, in some cases, alternate trees in the rows. Many of the lower branches on a large number of trees were completely defoliated and others bore only the young green needles of the current season's growth and those of the previous year (Fig. 1). These latter were of mature size but many of them were purplish brown and could be shaken easily from the twigs. The discolored needles bore small black superficial fruiting bodies that proved to be the pycnidia of *Rhizosphaera kalkhoffi* Bub. This fungus had not been reported previously in the United States but was known to occur on living needles of *Picea abies* (L.) Karst. in Bohemia and France (1), of *P. pungens* in Norway (2), and of *P. pungens* var. *argentea* Beiss. in Italy (1). Wilson and Waldie (7) reported it in Great Britain on species of *Picea*, and also on *Abies, Pinus*, and *Pseudotsuga*. Since 1938 *R. kalkhoffi* has been observed and collected in various localities in the eastern United States. It has been found only on spruce in this country, occurring on ornamental trees of *P. pungens* at Lakeville, Conn., Brooklyn, Peekskill, and Morris, N. Y., and Charlottesville, Va., and on species of *Picea* at Bar Harbor, Me., Falmouth, Mass., and Hamilton, Mass. In Canada it has been reported as abundant on *P. pungens* at Knowlton, Quebec (5). Its origin and the present extent of its distribution in North America are not known. It has never been reported on *P. pungens* in its native range.

The fungus seems to affect first the needles of the lowest branches and gradually progresses up the tree. The earliest indication of the disease, usually occurring late in the summer, is a yellow mottling of the mature needles of the current season's growth. In some of the discolored areas small tufts of brown thick-walled hyphae are found protruding from the stomata. Occasionally a few scattered needles on the newly developed twigs gradually become purplish brown and, in the spring, small black globoid pycnidia become evident above the stomata on all surfaces of the needles. The small white waxy mass that normally fills the stomatal opening adheres to the top of the pycnidium until it is mature. On severely diseased trees the infected needles usually fall during their second summer, leaving only the needles of the current season's growth.

[1] Associate Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, New Haven, Connecticut.

The pycnidia and spores on the infected needles corresponded with those of the type specimen[2] of Bubák's *Rhizosphaera kalkhoffi* and with his description of the fungus (1). The development of a pycnidium begins with the formation of a closely interwoven mass of hyaline and brown hyphae in the intercellular space below a stoma. A very few brown hyphae grow up through the stomatal cavity and branch out as they emerge above the stoma. By the repeated division of the cells of these hyphae the pycnidium is

Fig. 1.   Blue spruce tree affected with needle cast.   Photograph by H. G. Eno.

formed. The brown hyphae in the stomatal cavity between the guard cells develop into a well-defined stalk, characteristic of the genus (Fig. 2, A). The pycnidial wall is composed of a single layer of brown angular cells, enclosing the pseudoparenchymatous tissue from which the spore-bearing cells develop. The spores are produced histogenously by budding from any of the cells lining the pycnidial cavity. The mature pycnidia rupture irregularly and widely, exposing the spores, which are ovoid, unicellular, hyaline, with a slight brownish tinge in the wall at maturity, and measure $7-10 \times 3-5 \mu$.   No ascogenous stage has been observed.

[2] The writer is indebted to Dr. G. M. Reed, formerly Curator of Plant Pathology at the Brooklyn Botanic Garden, Brooklyn, N. Y., for the loan of Bubák's type specimen on file in that herbarium.

Before germination, the spores of *Rhizosphaera kalkhoffi* increase to about twice their normal size and, within 24 hours, send out germ tubes from both ends. The portion of the germ tube immediately adjacent to the spore soon becomes septate and a succession of short cells results. These cells branch profusely, forming a dense cluster of small-celled hyaline hyphae around the spore, which soon loses its identity in the rapidly growing hyphae
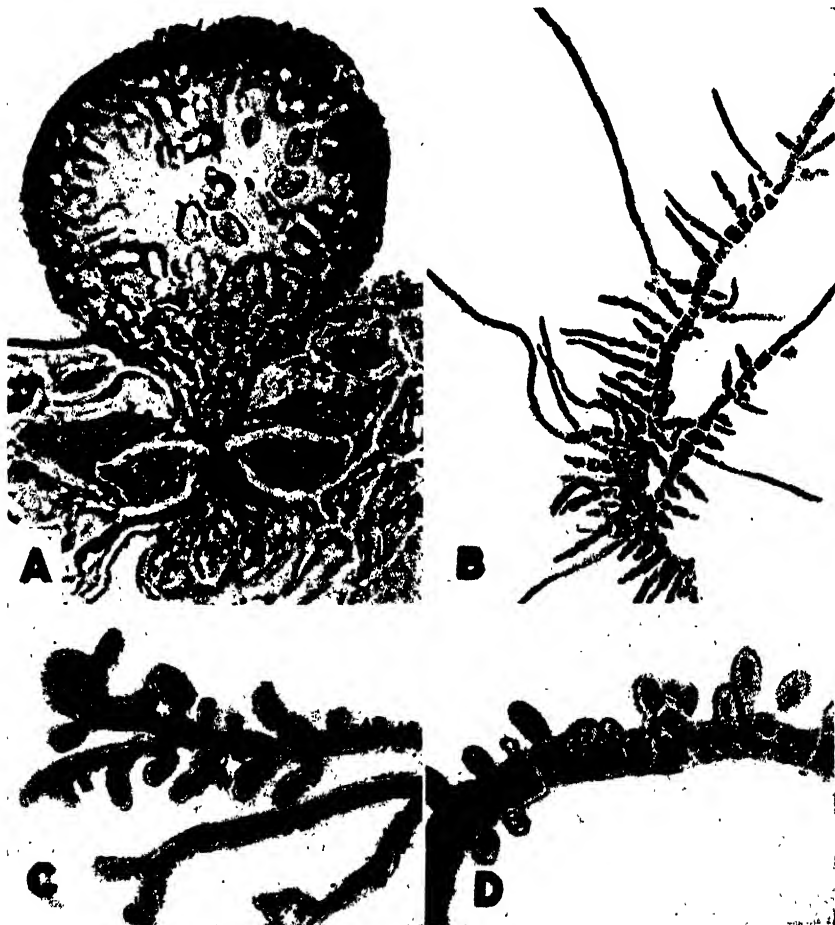
FIG. 2. A. Section through pycnidium of *Rhizosphaera kalkhoffi*, showing stalk in stomatal cavity. × 680. B. Germinating spore—2-day growth. × 165. C. Branched tip of hypha—10-day growth. × 165. D. Spores budding from hypha in culture. × 680. Photomicrographs by H. G. Eno.

(Fig. 2, B). The terminal cells of the hyphae are long, slightly enlarged at the tip, and filled with dense cytoplasm. After 10 days on Leonian's medium (3) in Petri-dish cultures some of the tips of the hyphae from a single spore become bulbous and thick-walled with a profusion of short branches (Fig. 2, C). Most of the hyphae except the growing tips eventually have thick walls, more or less knobby or uneven in thickness, and

change gradually from hyaline to brown. The hyphae resemble rather closely those found in the leaf tissue, particularly when the pycnidia are just beginning to be formed.

When grown on Bacto malt extract medium (4, p. 455) the color of the older mycelium in mass is at first "raw umber,"[3] and gradually darkens until it becomes black. Aerial hyphae are usually absent, both on the malt extract medium and on Leonian's medium. The advancing hyphae of a colony remain closely appressed to the surface of the medium and soon begin to produce an abundance of spores, which bud off in succession from any point along the hyphal cells (Fig. 2, D), and occasionally from the tips of short unicellular or long multicellular branches. The spores are loosely held together in gelatinous masses along the hyphae, giving a butyrous appearance to the surface of the culture. They are ovoid, unicellular, hyaline at first, with a brownish tinge in the wall at maturity, and are slightly wider than the pycnospores in nature, measuring $7\text{-}9.8 \times 4.4\text{-}6.6\ \mu$ (25 spores). In the older portions of the hyphae several adjacent sporulating cells divide and increase in width, with a thickening of the walls. The presence of these dividing cells and their gelatinous masses of spores in series at irregular intervals along a hypha give it a zigzag appearance. The cells gradually become greenish brown and finally dark brown and thick-walled, when spore production ceases. Cell division may continue until a large, more or less globoid mass of cells is formed. In some cases small hyaline cells are produced from the outer portion of these masses by division and are easily separated from the brown cells. They resemble the spores so closely that they cannot readily be distinguished from them in mass. No pycnidia have been found in culture.

No inoculations have been undertaken by any investigator to determine definitely the pathogenicity of *Rhizosphaera kalkhoffi*, its host range, and the particular conditions under which infection takes place. The presence of intercellular hyphae in the mesophyll of the leaf when the needles begin to show discoloration and the small tufts of hyphae in the stomata give evidence of the relation of the fungus to the disease. Infection seems to be confined to the needles. The uninjured terminal buds continue to develop new growth each year until the twigs become so weakened by premature defoliation that the new growth is stunted and the young needles are readily susceptible to infection. This results in a very slow progress of the disease throughout the tree. Even on severely diseased trees the growth of the unaffected branches in the upper portions of the trees continues at a normal rate. The significant damage is largely confined to the lowest branches and therefore decreases the value of affected trees for ornamental purposes. Wilson and Waldie (7), in Great Britain, reported the death of 14- to 20-year-old trees of *Picea pungens* resulting from defoliation by *R. kalkhoffi*, but no case of the death of entire trees from the needle cast are known in the United States.

[3] The descriptive color term is that given in Ridgway (6).

Satisfactory control of the disease on ornamental trees grown alone or in small groups may be obtained by spraying. Selected trees in the planting at Litchfield, Conn., were sprayed with 4–4–50 Bordeaux mixture, to which casein was added as a spreader. Three applications were made at intervals of two weeks, beginning the latter part of June when the needles of the new growth had developed. During the two seasons when the spraying experiments were carried on, the progress of the disease was arrested by the spray on those trees where infection had caused defoliation of some of the lowest branches. Neighboring trees that showed only slight infection before spraying were effectively protected from further spread of the disease.

DIVISION OF FOREST PATHOLOGY, BUREAU OF PLANT INDUSTRY,

SOILS, AND AGRICULTURAL ENGINEERING, AGRICULTURAL

RESEARCH ADMINISTRATION, U. S. DEPARTMENT OF AGRICULTURE,

IN COOPERATION WITH OSBORN BOTANICAL LABORATORY, YALE UNIVERSITY,

NEW HAVEN, CONNECTICUT.

LITERATURE CITED

1. BUBÁK, FR. Eine neue *Rhizosphaera*. Ber. Deut. Bot. Gesells. 32: 188–190. 1914.
2. JØRSTAD, I. Innberetning fra statsmykolog Ivar Jørstad om soppskydommer på skogtrærne i årene 1926–1930. Beretning om det Norske Skogwesen 1930: 78–96. 1931.
3. LEONIAN, L. H. A study of factors promoting pycnidium-formation in some Sphaeropsidales. Amer. Jour. Bot. 11: 19–50. 1924.
4. LEVINE, M., and H. W. SCHOENLEIN. A compilation of culture media for the cultivation of microorganisms. 969 pp. Baltimore, Md. 1930.
5. MACRAE, R., and I. L. CONNERS. Needle blight (*Rhizospha ra kalkhoffi* Bubák). Canadian Plant Disease Survey Ann. Rept. 20(1940): 87. 1941. Mimeo.
6. RIDGWAY, R. Color standards and color nomenclature. 43 pp. 53 color plates. Washington, D. C. 1912.
7. WILSON, M., and J. S. L. WALDIE. *Rhizosphaera kalkhoffi* Bubák, as a cause of defoliation of conifers. Royal Scottish Arbor. Soc. Trans. 40: 34–36. 1926.

FUNGI ASSOCIATED WITH RUNNER PEANUT SEEDS AND THEIR RELATION TO CONCEALED DAMAGE[1]

KENNETH H. GARREN AND B. B. HIGGINS[2]

INTRODUCTION

An internal discoloration and decomposition of runner peanut seeds that cannot be detected in unbroken seeds (Fig. 1), has become of considerable concern to peanut processors. This damage, generally known as "concealed damage" or "hidden damage," has necessitated the development of specific sampling methods by peanut brokers. At present many lots of peanuts offered for sale are graded down or rejected because of concealed damage.

The hyphal mats, which are usually found between the cotyledons of seeds with concealed damage (Fig. 1, B and C), indicate that the condition is a result of the action of filamentous fungi. As a first step in a general study of concealed damage, the fungi associated with runner peanuts in all conditions are being identified, and their connection with concealed damage is then being checked.

Investigations to date have revealed some definite relationships between species of fungi and damage. It seems advisable, therefore, to present an introductory report at this time.

REVIEW OF LITERATURE

All references to fungi associated with peanut fruits or to concealed damage to date have been in the form of short notes. Evans and Poole (1) in a report on fungi isolated from lesions on the seed coats and shells listed *Fusarium spp., Rhizoctonia solani* Kühn., *Rhizopus sp., Botrytis sp., Pythium sp., Sclerotium bataticola* Taub., and *S. rolfsii* Sacc. of the more common fungi, along with *Cephalothecium sp., Trichoderma sp., Penicillium sp.,* and *Aspergillus sp.* Prince (3) listed *Fusarium spp.* and *Alternaria spp.* as the predominant organisms associated with various bunch-peanut seeds, with *S. bataticola, S. rolfsii, Diplodia natalensis* Evans, *Trichoderma sp.,* and *Penicillium spp.* of moderate frequency. *R. solani, Rhizopus spp.,* and other fungi were isolated infrequently. The fungi noted by Prince were isolated from seeds, some of which appeared to have concealed damage. Concealed damage, however, is regarded as of little consequence in Spanish type peanuts (2).

Apparently the first published note on concealed damage was that of
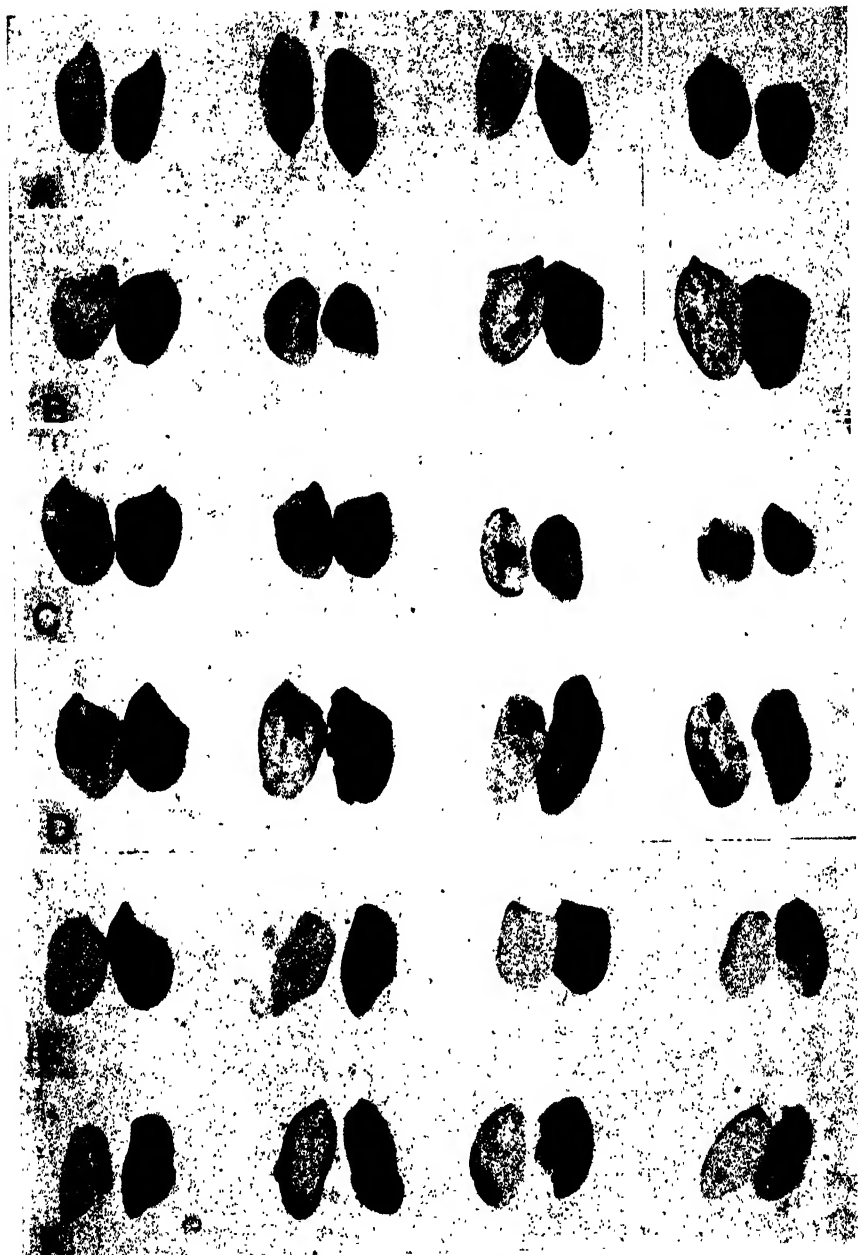
FIG. 1.   Concealed-damage seeds of Southeastern runner peanuts contrasted with sound seeds.   A. Prominent yellow discoloration of cotyledons, no fungus mat.   B. . Prominent mats of fungus hyphae between cotyledons.   C. Sclerotia of *Sclerotium batati-cola* in intercotyledonary space.   D. Late stage of concealed damage.   Discoloration of cotyledons faintly discernible through undamaged seed coat.   E. Sound seeds with un-damaged seed coats.   F. Seeds with discolored seed coats, cotyledons sound.

Rankin (4) in 1937 who found confectioners rejecting from 35 to 40 per cent of Georgia's 1936 runner peanut crop because of concealed damage. Some lots were rejected by oil mills. After examination and isolations Rankin concluded that concealed damage was caused by several soil fungi, particularly *Sclerotium bataticola* and *Rhizoctonia spp.*, that had penetrated the shells of the nuts when harvested plants were stacked in a wet, green condition.

Concealed damage has only recently received further attention. In 1944 Higgins (2) listed common molds and fungi such as *Rhizoctonia sp.*, *Sclerotium bataticola*, *Penicillium sp.*, and *Rhizopus sp.* as isolates from concealed damage seeds. Subsequently Wilson (5) isolated a *Diplodia sp.* from 65 per cent of concealed-damage seeds examined. Ninety-five per cent of Wilson's isolates were *Diplodia sp.*, with *S. bataticola*, *Fusarium spp.*, *Aspergillus sp.*, *Penicillium sp.*, *Rhizopus sp.*, and bacteria making up the remainder. On this basis Wilson considered the *Diplodia sp.* to be the primary organism associated with concealed damage. Later Wilson (6) found *Diplodia theobromae* (Pat.) Nowell the predominant organism in concealed-damage seeds, while *S. bataticola*, *Rhizoctonia sp.*, and miscellaneous molds and bacteria were associated with visibly damaged seeds. Thus, Wilson indicated that a *Diplodia sp.* alone is responsible for concealed damage.

In a recent publication Wilson (7) reported isolating the same molds and soil-borne fungi from young peanuts and regarded this as proof that the young peanut fruit is invaded by fungi soon after it penetrates the soil. In this same report Wilson stated that more than 80 per cent of the isolates from seeds in early stages of concealed damage are *Diplodia theobromae* with other fungi such as *Fusarium spp.* being isolated more frequently as concealed damage progresses. He regarded these fungi from seed in advanced stages of concealed damage as secondary invaders.

Evidence to date indicates that concealed damage is not found to any considerable extent in freshly dug peanuts. Some unpublished results from this laboratory showed no lots of peanuts with concealed damage at time of digging in 1945 and one of sixteen lots with concealed damage (2 per cent) at digging time in 1946. Results from Alabama (8) showed concealed damage in 13 of approximately 100 freshly dug lots in 1945. One lot showed 12 per cent concealed damage, one 3 per cent, and one 2 per cent, while the remaining ten lots all contained less than 1 per cent.

PROCEDURE

The nature and development of the peanut fruit suggests that many soil inhabiting fungi may be associated with the mature pod; thus, concealed damage may be assumed to develop as a result of the action of fungi normally associated with the fruit. This hypothesis has been tested by a series of laboratory studies leading up to flask inoculations as a final check on the relationship of various fungi to concealed damage.

Since this was not a detailed mycological investigation, most of the fungi

encountered were identified to genus only. The species of some fungi was obvious. When it appeared that more than one species of a genus had been isolated, the apparent species were labeled "isolate No. 1," "isolate No. 2," etc. Since the main concern was with fungi present in or on seeds, no isolations were made from shells.

The Southeastern runner variety (North Carolina runner) was used throughout.

1. *Identification of fungi associated with sound seeds.* In this study fruits were used from a combination of two lots of peanuts sun-dried immediately after plowing-out. Examination of 2,000 seeds from each lot revealed no concealed damage or visible damage of a fungus nature. Isolation of fungi was started approximately one month after harvest.

Approximately 200 fruits were surface sterilized by soaking them for two minutes in acidified 1 : 500 mercuric chloride solution and then washing them in sterile water for 5 minutes. The seeds were removed and split under aseptic conditions, and all seeds having exterior or interior discoloration or in which there was any possibility of outside contamination were discarded. One-half of each remaining seed was plated on sterile, acidified tap-water agar. Two hundred thirty-two half-seeds were plated.

2. *Identification of fungi associated with concealed-damage seeds.* Peanuts were obtained from 25 stacks in which nuts with concealed damage had been found. Approximately 150 apparently sound seeds from each stack were surface sterilized and split asceptically. Both cotyledons of 556 seeds with concealed damage were plated. Fungi grew rapidly from every seed plated.

3. *Identification of fungi associated with visibly damaged seeds.* Visibly damaged seeds were selected from ten seed-lots of various origins. Seeds in advanced stages of decay were discarded, and the remainder were surface sterilized, split aseptically, and plated. Fungi were isolated from 421 seeds.

4. *Inoculation tests on the relationship of various fungi to concealed damage.* In the procedure described, 17 fungi were isolated (Table 1). Each was used to inoculate peanut fruits. Isolates of *Sclerotium rolfsii* and *Pythium sp.* from peanut pegs (gynophores) were used also.

Fruits containing at least 50 seeds were surface sterilized and transferred aseptically to a flask containing the desired fungus growing on autoclaved winter pea seeds. The flasks were incubated at 25° C. for 4 to 40 days. At the end of each period fruits were removed, air dried, and the seeds removed. All seeds with visible damage were discarded. The remaining seeds were surface sterilized, split aseptically, and those with concealed damage were plated.

Two noninoculated lots were run as controls; one with surface sterilized fruits, one with fruits not sterilized.

5. *Determination of changes in fungus flora of seeds under conditions conducive to growth of fungi.* Surface-sterilized nuts from the sound lot were transferred aseptically to flasks containing autoclaved peanut cotyle-

dons. Seventy-five nuts were put in each flask. The substratum was intended as a source of both moisture and supplementary food.

These flasks were incubated at 25° C. for varying periods, after which the pods were removed and dried. The seeds were removed, surface sterilized, and split aseptically, and one cotyledon from each seed was plated. Occurrence of concealed damage and the various stages of visible damage was noted. The number of seeds thus plated always exceeded one hundred. Time intervals were 4, 7, 10, 20, 30, 35, and 40 days. As a control 20 fruits per time interval were incubated in individual test tubes on sterile, moist filter paper.

TABLE 1.—*Fungi isolated from sound and damaged runner peanut seeds*

Fungus	Percentage of seeds in indicated condition from which fungus was isolated		
	Sound—no damage	With concealed damage	With visible damage
Diplodia sp.	1.7	89.6	5.6
Sclerotium bataticola	1.0	3.5	14.9
Rhizopus sp.	11.2	1.6	21.0
Aspergillus spp. (2 isolates)	9.9		2.2
Penicillium spp. (2 isolates)	1.3	0.5	
Fusarium spp. (6 isolates)	1.3	0.7	5.0
Mucor sp.	0.9		1.0
Haplographium sp.	.	.	1.0
Rhizoctonia solani	.	0.9	. ..
Neocosmospora vasinfectum	a	. ..	.. ...
Sterile fungus	0.4	.	.....
Diplodia sp. in mixture	. .	1.6	.. ..
S. bataticola in mixture		0.7	. ...
Miscellaneous mixtures	. .	0.7	.
Bacteria	.	0.2	49.3
Nothing isolated ..	72.3	.	. ....

a *Neocosmospora vasinfectum* (Atk.) Smith was isolated from seeds after 7 days' incubation.

RESULTS

Table 1 gives the fungi isolated from seeds and the frequencies of these isolations. The results of inoculations to determine the relationship of eight of the species of fungi tested to concealed damage are presented in condensed form in table 2. The results for the other fungi tested were about the same as those for *Mucor sp.* and *Rhizopus sp.* with only *Diplodia sp.* and/or *Sclerotium bataticold* isolated from concealed-damage seeds.

With increasing length of incubation period for noninoculated fruits there were notable changes in the frequencies with which various fungi were isolated. The fungi that developed in peanuts incubated individually in test tubes were not significantly different from those that developed when pods were incubated together in flasks. Since this is regarded as indicating no appreciable spread of fungi from fruit to fruit, the results were combined and are given in table 3.

DISCUSSION

Fungi isolated from sound seeds removed aseptically from surface-sterilized pods were species usually encountered as saprophytes on dead plant tissue (*Sclerotium bataticola, Diplodia sp., Fusarium spp., Rhizopus sp., Aspergillus spp.*, etc.). Thus, living mycelium or spores of these fungi were present on the surface of the seeds, between the seeds and the shell, or within

TABLE 2.—*Results of inoculations of runner peanut fruits in flask cultures*

Inoculum and incubation period	Results		
	Concealed damage	Visible damage	Isolations from seeds with concealed damage
	per cent	per cent	Fungus — per cent
Control: surface sterilized fruits			
7 days' incubation	0	2	
10 do	16	8	S. bataticola — 73 / Diplodia sp. — 27
18 do	9	42	S. bataticola — 64 / Diplodia sp. — 36
Control: nonsterile fruits			
7 days' incubation	0	0	
10 do	3	4	S. bataticola — 66 / Diplodia sp. — 33
18 do	4	26	S. bataticola — 75 / Diplodia sp. — 25
Diplodia sp.			
7 days' incubation	12	4	Diplodia sp. — 100
10 do	44	14	do — 100
18 do	20	35	do — 100
Sclerotium bataticola			
7 days' incubation	11	6	S. bataticola — 100
10 do	28	7	do — 100
18 do	28	32	do — 100
Fusarium sp. No. 5			
18 days' incubation	21	49	S. bataticola — 90 / Fusarium sp. No. 5 — 10
Fusarium sp. No. 6			
18 days' incubation	8	53	Fusarium sp. No. 6 — 50 / Mucor sp. — 50
Neocosmospora vasinfectum			
18 days' incubation	25	2	S. bataticola — 66 / Rhizopus sp. — 33
Penicillium sp. No. 1			
18 days' incubation	14	26	Diplodia sp. — 80 / Rhizopus sp. — 20
Mucor sp.			
18 days' incubation	3	11	S. bataticola — 100
Rhizopus sp.			
18 days' incubation	16	7	Diplodia sp. — 75 / S. bataticola — 25

the seeds. Fungi, other than those isolated from sound seeds, must also be considered as members of the fungus flora of peanut fruits in curing stacks. For example, *Neocosmospora vasinfectum* and additional *Fusarium spp.* developed when sound fruits from the same lot were incubated; and one parasitic form, *Rhizoctonia solani*, was isolated from peanut fruits taken directly from stacks.

Influencing factors are too numerous to expect uniformity between localities or fields in regard to the fungus flora of peanut fruits. It should be anticipated, however, that some fungi will be found consistently because of their universal distribution in the soils of the peanut belt. A comparison of fungi isolated in this and other studies (1, 2, 3, 5, 6, 7) places *Rhizopus sp.*, *Sclerotium bataticola*, *Diplodia sp.*, and the species of *Fusarium* in this classification. Occasionally one of these anticipated species may not appear in a series of isolations, probably because of the action of such factors as antagonisms between soil fungi. Since Wilson (7) has found most of the fungi reported from mature peanut fruits also present in young peanuts soon after they penetrate the soil, it is apparent that these fungi are actively associated with the developing fruit over a considerable period of time.

TABLE 3.—*Changes in fungus flora of uninoculated peanut fruits under conditions conducive to growth of fungi, and the corresponding development of concealed and visible damage*

Fungus	Percentage of seeds from which fungus was isolated for various incubation periods							
	0 days	4 days	7 days	10 days	20 days	30 days	35 days	40 days
Nothing isolated	72.0	73.5	64.0	56.0	27.0	4.5	2.0	0.0
Rhizopus sp.	11.0	21.3	19.5	16.0	16.5	2.0	1.5	0.0
Diplodia sp.	1.8	2.4	4.0	7.0	10.5	2.3	1.0	0.0
Sclerotium bataticola	1.0	1.0	10.3	19.5	22.4	2.5	2.0	0.0
Fusarium spp.	1.3	0.6	0.4	1.0	1.9	2.5	2.0	3.5
Other filamentous fungi	12.9	1.2	1.8	0.5	9.1	20.2	26.0	32.0
Bacteria	0.0	0.0	0.0	0.0	12.7	66.0	65.5	64.5

/— No visible damage —/—— Visible damage ——/
/——— Concealed damage ———/   /—— Decay ——/

Isolation of the fungi listed in table 1 shows that there has been a general invasion of the pod by fungi. Study of concealed-damage seeds from field lots showed that the fungi concerned entered the intercotyledonary space, undoubtedly through the placenta, and spread over the surface of the cotyledons. Eventually there is considerable penetration of the cotyledons with accompanying discoloration and decomposition. On the other hand, almost all visible damage that can be attributed to the activity of fungi evidently results from the growth of fungi in the space between the shell and the seed. A good portion of this visible damage is damage to the seed coat only and is not accompanied by discoloration or decomposition of the cotyledons.

Once these fungi had invaded the shell, either in the soil or in the stack, there were, of course, two means of further development: one through the placenta into the intercotyledonary space, and the other from the shell into the space between the shell and the seed. The development of concealed damage in surface sterilized, noninoculated nuts (Table 2) shows that there can be an invasion of the intercotyledonary space by fungi commonly present and developing when conditions are favorable.

As far as species are concerned the fungi isolated from concealed-damage seeds were almost identical with those isolated from visibly damaged seeds and from sound seeds (Table 1). This indicates that much of the damage in each of the two types of damage is not only the result of the action of the same fungi but is also the result of the action of fungi normally associated with the mature pods. In addition, the results from noninoculated incubated pods (Table 3) show that concealed damage may eventually become visible damage. Thus, as previously indicated by Wilson (6), concealed damage is one of the early stages in decay and under proper conditions develops into visible damage. This does not mean, of course, that all decay or other fungus-produced visible damage results from preceding concealed damage. It is probable that only a very small percentage of the evident decay or other visible damage found in mill-run runner peanuts had its beginning in concealed damage.

It is evident from table 2 that numerous competitions and antagonisms develop when peanut fruits with their natural fungus flora are inoculated with specific fungi. Certainly *Diplodia sp.* and *Sclerotium bataticola* were the more vigorous in these competitions since they developed in almost all flasks regardless of the inoculum used. Moreover, when *S. bataticola* was used as inoculum, no other fungus was isolated from any of the damaged seeds. Results given in table 2 indicate that *Rhizopus sp.* and *Mucor sp.* and certain isolates of *Fusarium sp.* are sometimes capable of considerable development in competition with other fungi. As suggested by the results from flask inoculations with *Fusarium sp.* (isolate No. 6), *Neocosmospora vasinfectum*, and *Penicillium sp.* (isolate No. 1) other antagonisms might result in reduced activity by either *Diplodia sp.* or *S. bataticola.* •Competitions and antagonisms similar to those developing in the inoculated flasks undoubtedly are active in field soils and in stacks of curing peanuts. This would explain the preponderance of isolations of *Diplodia sp.* and *S. bataticola* from concealed-damage seeds from the field.

As demonstrated herein, conclusive tests to determine which fungi cause concealed damage are difficult because of the presence of various fungi in sound fruits. This difficulty is emphasized in the inconsistent results given in table 2 for most fungi used. These results, however, may be taken as demonstrating that both *Diplodia sp.* and *Sclerotium bataticola* may produce concealed damage. More concealed-damage seeds developed in sterilized fruits inoculated with these fungi than developed in any of the other lots, and the fungus used in inoculation was reisolated from all of these seeds. The connection of these fungi with concealed damage is further emphasized in table 3 where the development of concealed damage in noninoculated fruits coincided with the period of major development of *Diplodia sp.* and *S. bataticola.*

For the 1945 season *Diplodia sp.* (Table 1) was by far the most important cause of concealed damage in the fields sampled. The action of *Sclerotium bataticola* in the inoculation tests show that in other seasons, in

other fields, or under varied conditions *S. bataticola* might equal or exceed *Diplodia sp.* in producing concealed damage. It should be noted that both Rankin's (4) and Higgin's (2) lists of fungi isolated from concealed-damage seeds included *S. bataticola* but did not include *Diplodia sp.*

There is no reason, however, for excluding other fungi from consideration as possible agents in the production of concealed damage. These inoculation tests show only that under the conditions in the flasks *Diplodia sp.* and *Sclerotium bataticola* are capable of producing concealed damage and also are capable of outgrowing or suppressing most of the other fungi that were associated with the peanut fruits. Since concealed damage results from saprophytic action, and since several fungi are associated with peanut fruits, it is evident that the action of fungi other than *Diplodia sp.* and *S. bataticola* may result in concealed damage. There were several concealed-damage seeds from which fungi other than *Diplodia sp.* and *S. bataticola* were the only organisms isolated (Table 1). *Diplodia sp.* and *S. bataticola* grow so rapidly under the conditions used for isolations it seems certain that they were not present in these particular seeds. The damage, therefore, could have resulted only from the action of the fungi isolated. In addition there is further evidence in table 2 that other fungi produce concealed damage. Each of two isolates of *Fusarium sp.* (isolates No. 5 and No. 6) were reisolated from some concealed-damage seeds after being used as inoculum. The high percentage of isolates of *Rhizopus sp.* and *Mucor sp.* from seeds from certain flasks indicates that these two fungi also may produce the condition, even though neither was reisolated from concealed-damage seeds when used as inoculum.

Of the fungi found to be associated with peanut fruits *Rhizopus sp.*, *Diplodia sp.*, and *Sclerotium bataticola* are capable of making the most rapid initial growth (Table 3). Thus, when curing peanuts are exposed to conditions favorable to fungus growth, the initial saprophytic activity of these three fungi should result in some concealed damage. Prolonged exposure to such conditions should bring other filamentous fungi and bacteria into action and result in further concealed damage followed by complete decay. Such a sequence of fungi is evident in table 3 where the other fungi appear to be "post-saprophytes" in that their period of major activity follows that of the three fungi mentioned.

Fungi capable of producing concealed damage have been demonstrated to be present in the fungus flora of peanut fruits when taken directly from the soil (Table 1). The fact that concealed damage develops in surface sterilized, noninoculated fruits when incubated (Table 2) shows that concealed damage may result from the action of fungi normally associated with the mature fruit. Consequently, any control methods must be aimed, not at keeping fungi away from curing peanuts, but rather at preventing the development of fungi already present. Work now in progress at this Station and in Alabama (8) indicates that the development of concealed damage may be reduced significantly by certain curing methods.

SUMMARY AND CONCLUSIONS

In a general study of concealed damage, the fungi associated with peanut fruits are being identified and tests are being made to determine which can produce concealed damage.

The fungi most frequently associated with peanut fruits are common soil-borne saprophytes such as *Rhizopus sp., Aspergillus spp., Sclerotium bataticola, Diplodia sp.,* and species of *Fusarium.* Species parasitic on peanuts are uncommon.

Uniformity between localities or fields in fungus flora of peanut fruits is not to be expected. Although certain prevalent fungi, such as *Diplodia sp., Sclerotium bataticola,* and *Fusarium spp.,* appear consistently in this flora, antagonisms or other factors may result in the temporary elimination of one or more of these prevalent fungi.

Concealed damage results when a fungus invades the intercotyledonary space through the placenta, while most of the visible damage of a fungus nature results from the activity of fungi in the space between the shell and the seeds. A comparison of fungi isolated from visibly damaged seeds, from concealed-damage seeds, and from sound seeds indicates that much of the damage in each of the two types of damage is the result of the action of the same fungi, and that these fungi are normally associated with runner peanut fruits.

Antagonisms and competitions develop when peanut fruits with associated fungi are inoculated with a specific fungus. *Diplodia sp.* and *Sclerotium bataticola* were more vigorous in these artificially induced competitions and usually outgrew any other fungus used as inoculum. Similar antagonisms and competitions developing in soils and in curing stacks would account for the preponderance of *Diplodia sp.* and *S. bataticola* in isolations from concealed-damage seeds from fields.

Inoculation tests have shown that both *Diplodia sp.* and *Sclerotium bataticola* can produce concealed damage. There is evidence, however, that several other species of fungi associated with peanut seeds can produce this condition. Laboratory tests on these other fungi are made difficult by the presence of various fungi in the peanut fruit.

The initial growth of *Rhizopus sp., Diplodia sp.,* and *Sclerotium bataticola* was more rapid than that of the other fungi associated with peanut seeds. When harvested peanuts are exposed to conditions favorable for the growth of fungi, concealed damage may result from the early saprophytic activity of these fungi.

Since fungi capable of producing concealed damage are associated with peanut fruits when the crop is taken from the soil, control methods for concealed damage are limited to preventing the growth of these fungi during the curing process.

DEPARTMENT OF BOTANY,
   GEORGIA AGRICULTURAL EXPERIMENT STATION,
      . EXPERIMENT, GEORGIA.

LITERATURE CITED

1. EVANS, M. M., and R. F. POOLE. Some parasitic fungi harbored by peanut seed stocks. (Abstr.). Jour. Elisha Mitchell Sci. Soc. **54**: 190–191. 1938.
2. HIGGINS, B. B. "Concealed damage" of runner peanuts. (Mimeo.). Georgia Agr. Exp. Sta. Press Bull. 536. 1944.
3. PRINCE, A. E. Fungi isolated from peanuts collected in South Carolina in 1944. U. S. Dept. Agr., Pl. Dis. Reptr. **29**: 367–368. 1945.
4. RANKIN, H. W. Concealed damage of runner peanuts in Georgia. U. S. Dept. Agr., Pl. Dis. Reptr. **21**: 30. 1937.
5. WILSON, C. A *Diplodia* associated with concealed damage in peanuts. Phytopath. **35**: 480. 1945.
6. ——————. Damaged peanuts in Alabama in 1945. U. S. Dept. Agr., Pl. Dis. Reptr. **30**: 12–13. 1946.
7. ——————. A survey of the fungi associated with peg and seed rots of peanuts in southern Alabama. (Abstr.). Phytopath. **37**: 24. 1947.
8. ——————, and J. P. WILSON. Field studies on the relationship between the method of curing and the development of concealed damage in runner peanuts. U. S. Dept. Agr., Pl. Dis. Reptr. **29**: 61–63. 1945.

PHYTOPATHOLOGICAL NOTES

An Unusual Case of Clitocybe Root Rot in Ficus elastica Propagation Stock in a Florida Nursery.[1]—One of the most remarkable cases of mushroom root rot caused by *Clitocybe tabescens* (Scop. ex Fr.) Bres. that the writer has seen in 20 years' observations on this disease in Florida was found in a stock planting of India-rubber fig (*Ficus elastica* Roxb.) and the variety *variegata* grown, for propagation of the tips for the northern trade, under a lath-shed at a large nursery near Apopka, Florida. The planting comprised nearly three-fourths of an acre of closely set stock plants. These had been planted about 2½ years and were about a year old when set out. To induce rooting, the stems back of the growing tips were packed with sphagnum moss before the tips were severed for potting. The plants were watered every day by means of overhead irrigation in order to keep the moss wet. About 6 weeks are required for the "mossed" branches to develop a good root system and after the crop is cut the treatment is repeated when new shoots develop. From 2 to 3 crops are thus secured a year.

Clitocybe root rot had been observed in this planting for about two years by Mr. John R. Springer, nursery inspector of the State Plant Board of Florida, to whom the writer is indebted for calling his attention to this case. The disease was said to be becoming progressively worse and it was estimated to have killed 20–25 per cent of the original stock planting, much of which had been reset from time to time, as well as some of the newly set stock. When this planting was examined with Mr. Springer on June 2, 1944, a large number of plants were found to be more or less completely girdled by the root-rot fungus and a considerable number of dead ones had been pulled up shortly before but not yet carried out. In some cases completely girdled plants continued to live because they had developed adventitious roots above the girdled bases. In many instances fresh clusters of the sporophores of *C. tabescens* had developed from the bases of attacked plants. The disease occurred scattered throughout a large part of the planting but was much worse on the higher portion, the planting being on a slope. Some idea of the loss occasioned by this disease may be secured from the fact that rooted tips were worth 20 cents apiece wholesale at the time and that an average of 5 rooted tips were secured a year from each plant.

The practice of sprinkling the plants daily to keep the moss layers wet unquestionably was highly conducive to infection and the extremely rapid development and spread of the disease. Owing to the sandy nature of the soil, however, there was no tendency to waterlogging.

By January, 1947, the disease had become so severe that growing these plants in this particular location was no longer profitable, and the small

[1] These observations were made while the writer was employed in Florida on the Emergency Plant Disease Prevention Project of the U. S. Department of Agriculture. The writer is at present Biologist, Industrial Test Laboratory, Philadelphia Naval Shipyard.

number remaining had been destroyed and other crops planted. The new crop of ferns does not appear to be injured by the fungus.

It is of interest that one plant enthusiast, who was troubled by frequent losses of cherished plants from Clitocybe root rot over a period of several years at his place at Lake Alfred, Florida, reported that after he discontinued watering his plants so frequently during dry weather when getting ready to move them to a new locality, he experienced a marked reduction in losses from this disease.

The efforts of the writer in making inoculations with *Clitocybe tabescens* in past years in Florida were severely handicapped by the prevalence of droughty soil conditions, which tended to make results very slow and uncertain. The exceptionally rapid spread and destructiveness of this fungus in the planting described above suggests that overhead irrigation or some other method of maintaining a high moisture content of the soil should prove of great value in conducting inoculation experiments.—ARTHUR S. RHOADS.

Breeding Wheat to Combine Resistance to Leaf Rust, Speckled Leaf Blotch, and Glume Blotch.[1]—The acreage of wheat in Arkansas has been reduced during the past decade or two primarily because of the prevalence of leaf rust (*Puccinia rubigo-vera* var. *tritici*), speckled leaf blotch (*Septoria tritici*), and glume blotch (*S. nodorum*).

In 1941 a start was made to breed wheat for disease resistance, not primarily for bread or pastry wheats but for winter pasturage and grain feed. Among the various wheats under study at the university farm, Fayetteville, was Fultz Sel. × Hungarian, C.I. 12017. It was noted that while this wheat had less leaf rust than any named variety, nearly all the plants had a few relatively large pustules (3-4 type) indicative of a susceptible reaction. However, a few plants with no such reaction appeared not only highly resistant to leaf rust but had fewer infections of *Septoria tritici* and *S. nodorum*. Of many varieties and selections of wheat under study, this was the first that seemingly had a combined resistance to the 3 diseases although its resistance to *S. tritici* was not of a very high type.

Acting on the theory that C.I. 12017 was not homozygous for resistance to these diseases and that the few plants noted as having no large leaf-rust pustules might represent segregates of a more resistant type, 6 single head selections were made from these in 1943.

Since the plants derived from these single head selections again lacked uniformity in disease resistance in 1944, single heads or single plants were selected once more in 1944, and in the succeeding generation in 1945. Thus three successive series of selections were made.

Compared with C.I. 12017 most of these selections, present in one or more rod rows in 1946, had a higher and more uniform type of resistance to leaf rust and to glume blotch, and somewhat more resistance to speckled leaf blotch. However, while some of these selections appeared uniformly resis-

tant, others were apparently still segregating. Of 37 such selections, 25 had a lower and more uniform rust reading than the original while 12 showed no increase in resistance. On April 25, when Purplestraw (Alabama Bluestem), which was used as a check every tenth row, and Sanford, which in former years had been resistant to leaf rust, gave readings of 35 to 50 per cent leaf rust, none of the selections of C.I. 12017 had more than a trace. On May 18–20, when Purplestraw and Sanford had fully 90 per cent of their upper blades occupied by leaf rust pustules and when their lower blades were dead, killed mostly by combined action of speckled-leaf-blotch and leaf-rust fungi, the highest reading obtained on the original C.I. 12017 and its selections was 20 per cent. Most of the selections had merely a trace of leaf rust, the pustules being few, very small, and of a type that led one to consider the selections intermediate between resistant and susceptible. However, the presence of a large number of chlorotic flecks on these selections without any pustules indicated a high type of leaf-rust resistance.

TABLE 1.—*Comparison between Selection 12017–24–2, C.I. 12017, and Sanford wheat in reaction to 8 race groups of the leaf-rust fungus*

Wheat selection or variety			Reaction[a] to race group:[b]					
			6	9	12	21	45	65
12017–24–2	X	X	R	R	R	R	R	R
C.I. 12017	S,X	S,X	R	R	R	X,R	S,R	R
Sanford	R,S	S	S	S	R	S	R	S

[a] Key: S = susceptible, R = resistant, X = intermediate or indeterminate, S,X; S,R; etc. = mixture of wheats with different reactions indicating varietal impurity or segregating condition.

[b] Chester combines a number of formerly recognized races into a race group; he includes in race group 2 the former races 2, 3. 15, 25, 34, 59, 62. 102, and 127; in group 5 he includes races 5 and 52; in group 6 he includes races 6, 28, 39, 105, and 126; in group 9 he includes races 9, 10, 13, 19, 20, 24, 27, 29, 31, 108, and 115; in group 12 he includes races 12, 32, 44, 58, 61, 76, 81, 84, 85, 88, and 90; in group 21 he includes races 21, 30, 35, 42, 54, 77, 89, 94, and 122; in group 45 he includes races 45, 57, and 67; and in group 65 he includes races 65, 80, and 101.

Since K. Starr Chester of the Oklahoma Agricultural and Mechanical College had very kindly offered to determine leaf-rust reaction to specific races, seed of the selection 12017–24–2 were sent to him along with seed of C.I. 12017 and of Sanford. The selection sent, while not having the highest type of leaf-rust resistance in 1946, leaf-rust infection having been read as 15 per cent on May 20, was the only one for which seed were available at the time of his testing. His results are presented in table 1.

While C.I. 12017 gave both a susceptible and an intermediate, or a susceptible and a resistant reaction to race groups 2, 5, 21, and 45, selection 12017–24–2 yielded a uniformly higher type of resistant reaction to all 4 race groups (Table 1). This is consistent with the field behavior of this selection and some of its sister selections.

Chester notes that 12017–24–2 is particularly interesting because of its high resistance to race group 21. He finds all 5 differential varieties which he uses in identifying races of leaf rust[2] are susceptible to this race group, indicating a wider degree of aggressiveness for this race group than for any of the others. Although he seemingly did not find this race group common in 1946[3] or previous years, it represents a threat to any susceptible variety where leaf rust is commonly destructive. The resistance of 12017–24–2, a soft red wheat, to race group 21 appears to be of some importance to future breeding work.

Aside from their resistance to leaf rust and partial resistance to the two Septorias, these selections were not injured by Hessian fly when planted early in the fall of 1945 and of 1946, a month prior to the fly-free date. The selections seem to possess more winter hardiness than Sanford, Sanett, Austin, and Purplestraw, and suffered much less winter injury during the severe freeze of December 29, 1946, to January 6, 1947. Their grain yields appear promising. Straw characters remain largely to be determined although they appear to be desirable. Most of these selections are beardless.

According to R. M. Caldwell (personal communication), C.I. 12017 represents a selection from the cross Fultz selection C.I. 11512 × Hungarian C.I. 4830–1, the cross having been made in 1929 by E. B. Mains and L. E. Compton at the Agricultural Experiment Station, Purdue University. F_2 seed were turned over to Caldwell in 1930 and he, with the help of Compton, made selections, the last one in the F_6 generation. Caldwell notes that the main genes for resistance to leaf rust were derived from Fultz C.I. 11512. This is a sister line to Wabash, both having been derived from a segregating line of Fultz C.I. 5308. He further notes that C.I. 12017 is more resistant than the Fultz parent and derives resistance to some races from the Hungarian parent (a synonym of Turkey according to Clark and Bayles[4]) from which it also inherited resistance to most races of bunt. However, C.I. 12017 is very susceptible to loose smut. Otherwise, according to Caldwell, it would have been released.

No adequate tests or significant field observations have been made of the selections here described relative to resistance to bunt or susceptibility to loose smut. Both of these diseases are usually of minor importance in Arkansas. Nevertheless, large increase in acreage of any wheat variety or of any other crop would probably give rise to pathological problems that are of no great importance at present.

Another breeding line that appears to offer hope of combined resistance to leaf rust and the two Septorias is represented in a cross (Red Rock × Hope) × C.I. 12017. The Red Rock × Hope parent represents a cross that was made by E. B. Mains in 1927 at the Agricultural Experiment Station,

[2] Chester, K. Starr. The nature and prevention of the cereal rusts as exemplified in the leaf rust of wheat. 269 pp. Chronica Botanica, Waltham, Mass. 1946.

[3] Chester, K. Starr, and D. A. Preston. Another epiphytotic of fall wheat leaf rust in the southwest. U. S. Dept. Agr., Pl. Dis. Reptr., 31: 15–17. 1947.

[4] Clark, J. Allen, and B. B. Bayles. Classification of wheat varieties grown in the United States in 1939. U. S. Dept. of Agr., Tech. Bul. 795. 1942.

Purdue University, and selected out by R. M. Caldwell and L. E. Compton of the same institution (personal communications from R. M. Caldwell and C. O. Johnston). It was further reselected by C. O. Johnston at Manhattan, Kansas, and placed in the uniform winter wheat rust nurseries in 1937. In 1941 and 1942, when leaf rust and speckled leaf blotch were severe at the university farm, Fayetteville, some plants of this cross were freer from these two diseases than most of the other plants. Fifteen single head selections were made from the resistant plants and their progeny grown from 1942 to 1946.

These selections while having fair resistance to *Septoria tritici* seemingly developed more and more leaf rust during these years. This also happened to most Hope crosses, the variety Austin being the exception, and it was probably due to the greater prevalence of races to which these Hope crosses were susceptible. In any case, selections from Red Rock × Hope were so badly rusted in 1946 that they are being discarded.

The cross (Red Rock × Hope) × C.I. 12017 was made by the writer in a greenhouse during the winter of 1942–43. The F₁ grown outdoors seemingly had a high degree of resistance to leaf rust and speckled leaf blotch. Not enough glume blotch was present to judge of resistance to this disease.

Single plant selections have been made in the F_2 and F_3, the current generation being the F_4, the selections again being made primarily for resistance to the 3 diseases. Since the lines are still segregating, there is no assurance that resistance to these diseases will be combined with other desirable characters. However, there appears to be little doubt that a higher type of resistance to *Septoria tritici* has been obtained in some of the selections coupled with a type of reaction to leaf rust that approaches immunity. In this type of reaction there are few or no chlorotic or necrotic flecks and a complete absence of pustules.

The cross was made with the hope of combining the higher type of resistance to speckled blotch to be found in the Red Rock × Hope parent with the leaf rust resistance, head characters, and better straw of C.I. 12017. It was also hoped that the stem rust resistance of Red Rock × Hope might be incorporated into this cross although this disease is usually not important in Arkansas. Up to the present there are insufficient data to indicate that this has been accomplished.—H. R. ROSEN, Arkansas Agricultural Experiment Station, Fayetteville, Arkansas.

A Pythium Plate Method of Evaluating Fungicides.—A supplement to other methods for evaluating fungicides has been developed with *Pythium debaryanum* Hesse as the test organism.[1]

The fungus is grown on potato-dextrose agar (Difco prepared) for 5 days. A piece of the infested agar, one-half inch square, is placed in the center of a sterile Petri dish and seeded with approximately 50 sterilized wheat seeds. Five ml. sterile water were added to maintain sufficient mois-

[1] In the early stages L. Shanor contributed to developing this technique.

ture.　The plates are held for 4 days at 24° C., by which time each seed is thoroughly infested.

Twenty ml. of the fungicide solution is then poured into a 10-cm. Petri dish and one infested wheat seed is placed in the center of the dish.　Measurements of mycelial growth are made after 48 hours.　A special glass-topped box containing a 60-watt electric bulb with a millimeter rule attached to the surface of the glass top was used for this purpose.　Each plate is placed on the ruler and the distance from the seed to the perimeter of the mycelial growth is recorded.　Plates containing 20 ml. distilled water and one infested seed are used as controls.

Ten compounds, including 8-quinolinol and 9 of its derivatives, were tested by this method.　The same compounds were evaluated by the settling tower method of McCallan[2] and by the spore-dilution-slide method[3] using *Stemphylium sarcinaeforme* (Cav.) Wilts. as the test organism.

A comparison of the 10 compounds evaluated by the 3 procedures is shown in table 1.　The *Stemphylium* spore-dilution method and the *Pythium*

TABLE 1.—*Rank of ten compounds evaluated by three laboratory assay procedures, employing two test organisms*

Compound	Method of assay				
	Stemphylium		*Pythium*		
	Tower	Dilution	Plate tests		
Copper 8-quinolinolate	1	1	1	1	1
Magnesium 8-quinolinolate	2	3	4	4	4
8-quinolinol	3	5	2	3	3
Zinc 8-quinolinolate	4	8	8	8	10
Manganese 8-quinolinolate	5	4	7	6	6
5,7, dibromo 8-quinolinol	6	6	5	5	5
5,7, dichloro 8-quinolinol	7	2	3	2	2
Copper, 5,7, dibromo 8-quinolinolate	8	7	6	7	7
Iron 8-quinolinolate	9	9	9	9	8
5,7, dinitro 8-quinolinol	10	10	10	10	9

plate method correspond favorably; differences between 2 test organisms of the degree shown in the table are not unusual.　The 3 procedures show the same trend of toxicity.　Since the *Pythium* plate method is simple and time saving, it should prove useful in screening compounds as fungicides.— CURTIS L. MASON and DWIGHT POWELL, Division of Plant Pathology, Department of Horticulture, University of Illinois, Urbana, Illinois.

Influence of Fasting in the Transmission of the Beet-Mosaic Virus by the Green Peach Aphid.—Watson[1] published data which indicated that a period

[2] McCallan, S. E. A., and Frank Wilcoxon.　An analysis of factors causing variation in spore germination tests.　II.　Methods of spraying.　Contrib. Boyce Thompson Inst. 11: 309–324.　1940.

[3] The American Phytopathological Society, Committee on Standardization of Fungicidal Tests.　The slide-germination method of evaluating protectant fungicides.　Phytopath. 33: 627–632,　1943.

[1] Watson, M. A.　Further studies on the relationship between *Hyoscyamus* virus 3 and the aphid *Myzus persicae* (Sulz.) with special reference to the effects of fasting.　Proc. Roy. Soc. Lond., B. 125: 144–170.　1938.

of fasting prior to a short infection feeding resulted in an increase in vector efficiency in the transmission of a nonpersistent[2] virus. The virus used was Hyoscyamus virus 3 and the vector was the green peach aphid, *Myzus persicae* (Sulzer). Watson and Roberts[2] and Watson[3] have since published additional data regarding this phenomenon, and the results have been accepted generally in respect to validity. The evidence of Watson has been substantiated in England by another worker,[4] but to the knowledge of the author it has lacked confirmation among the virus workers in the United States.

Recently it has been possible to demonstrate the action of a period of fasting prior to an infection feeding, using the beet-mosaic virus, as the example of the nonpersistent virus, and the green peach aphid as the vector.

TABLE 1.—*Results[a] of trials to determine the influence of a fasting period prior to an infection feeding upon the ability of the green peach aphid to transmit the beet-mosaic virus*

Replication	Vector treatment	Infection feeding in minutes			Totals
		0.5	1.0	2.0	
A	Fasted	0/5	2/5	0/5	2/15
	Unfasted	0/5	0/5	0/5	0/15
B	Fasted	0/5	1/5	1/5	2/15
	Unfasted	0/5	0/5	0/5	0/15
C	Fasted	0/5	1/5	0/5	1/15
	Unfasted	0/5	0/5	0/5	0/15
D	Fasted	1/5	0/5	2/5	3/15
	Unfasted	0/5	0/5	1/5	1/15
E	Fasted	1/5	2/5	2/5	5/15
	Unfasted	2/5	0/5	0/5	2/15
Totals	Fasted	2/25[b]	6/25	5/25	13/75
	Unfasted	2/25	0/25	1/25	3/75

χ^2 6.98 for 1 degree of freedom.

[a] In the ratios listed, the numerator is the number of plants infected, while the denominator is the number of plants inoculated.

[b] In a second trial, using 6 replications, 5 plants per replication, the infection ratios obtained were: fasted, 15/30; unfasted, 2/30.

Apterae (wingless females) obtained from a noninfective colony of the green peach aphid established upon a sugar beet in the greenhouse were fasted in a vial for a minimum of 2 hours and a maximum of 4 hours. Following the completion of the starvation period, the infection feedings were begun. Single insects were fed upon a single virus source for infection feeding intervals of 0.5, 1.0, and 2.0 minutes. Five single aphids were fed

[2] Watson, M. A., and F. M. Roberts. A comparative study of the transmission of Hyoscyamus virus 3, potato virus Y and cucumber virus 1 by the vectors *Myzus persicae* (Sulz.), *M. circumflexus* (Buckton), and *Macrosiphum gei* (Koch). Proc. Roy. Soc. Lond., B, 127: 543–576. 1939.

[3] Watson, M. A. The transmission of beet mosaic and beet yellow viruses by aphids; a comparative study of a non-persistent and a persistent virus having host plants and vectors in common. Proc. Roy. Soc. Lond., B, 133: 200–219. 1946.

[4] Kassanis, B. Transmission of tobacco etch viruses by aphids. Ann. Appl. Biol. 28: 238–243. 1941.

for each interval and then placed singly upon healthy beet seedlings for a 24-hour test feeding period. During the same day single apterae from the colony were selected and used in a like manner, with the exception that they were not fasted prior to the infection feedings.

The procedure was repeated in order to obtain 5 replications of each infection feeding interval with a comparison between previously fasted and unfasted individuals. The results are given in table 1.

The total number of infections obtained under the influence of fasting apparently was significant over the total obtained by unfasted vectors. However, the relationship was not demonstrated by the 0.5-minute interval. In order to test this interval further, 6 replications, using 5 plants in each replication, comparing fasted and unfasted apterae at the 0.5-minute interval, were made. In these trials, 2 out of the 30 plants became infected in the unfasted series, while 15 out of the 30 plants became infected in the fasted series.

The data indicate that the factor of fasting has a beneficial effect upon the ability of green peach aphid apterae to transmit the beet-mosaic virus, when used in conjunction with short infection feeding periods. Consequently, it is confirmatory evidence for the phenomenon initially described by Watson in connection with the Hyoscyamus virus 3 and subsequently for a number of other nonpersistent viruses.—E. S. SYLVESTER, Division of Entomology and Parasitology, University of California, Berkeley, California.

Berg's Rust-resistant Red Cedar Susceptible to Phomopsis juniperovora in Greenhouse Tests.—In 1940 Berg[1] reported a red cedar (*Juniperus virginiana* L.) clone highly resistant to cedar-apple rust caused by *Gymnosporangium juniperi-virginianae* Schw. The clone had been observed for 16 years in the field under conditions of natural infection and for an additional period of 4 years' exposure to rust under more closely controlled field conditions. The writer became interested in the Berg cedar with regard to its possible resistance to *Phomopsis juniperovora* Hahn[2] which causes the destructive blight of nursery cedars. In a letter to the writer Berg stated that from his field observations the rust-resistant cedar did not seem to be resistant to *Phomopsis*. In the spring of 1941 he generously provided the writer with 4 small rust-resistant red cedar grafts for controlled testing. The Berg clone was first cultivated in pots in an unheated greenhouse. Two of the plants died; the two remaining plants, which at first grew slowly, were transplanted finally to a floor bed in the same house, where they have grown vigorously, producing foliage of a very handsome bluish-green color.

The two rust-resistant cedars were inoculated in 1946 with an isolate of *Phomopsis juniperovora* taken originally from a diseased branchlet of a 25-year-old red cedar hedge at Manhattan, Kansas. This material had been collected in January, 1945, by C. M. Slagg and after storage in an ice box at

[1] Berg, A. A rust-resistant red cedar. Phytopath. **30**: 876–878. 1940.
[2] Hahn, G. G. Taxonomy, distribution, and pathology of *Phomopsis occulta* and *P. juniperovora*. Mycologia **35**: 112–129. 1943.

approximately 40° F., a number of monospore isolations of the parasite were made by the writer the following September. Isolate No. 1 of this series was used in October, 1945, to inoculate a susceptible red cedar. A reisolation of this isolate, made in August, 1946, was utilized again the following month for 8 slit-wound inoculations of the two trees of the Berg clone. The inoculations were made according to a method previously described,[3, p. 903] four to each tree, with accompanying checks. Infections resulted from all inoculations; the checks were not infected. Lateral branches, 3 to 4 mm. in diameter, inoculated close to the trunk, subsequently died back to the main axis. When inoculum was placed on the trunk, which was 7 mm. in diameter at the point of incision, the parasite girdled the trunk and slowly killed the terminal. However, the pathogen attacked tissue for only 2 cm. below the inoculation site. Within two months erumpent sporulating pycnidia were observed about the inoculation incisions. Reisolations in early December of *P. juniperovora* from diseased inner-bark tissue produced on Bacto malt extract agar[4, p. 455] yellow coloration and red crystals in the medium, two cultural characteristics that were first indicated[5] for the parasite 30 years ago and that proved to be specific. These constant characters have facilitated accurate identification of the species since its discovery.

Under the conditions of the test the Berg rust-resistant red cedar clone was not resistant to juniper blight. Moreover, the results indicate that the basis of resistance to the rust in this clone is not operative with respect to *Phomopsis juniperovora.*—GLENN GARDNER HAHN, Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, in cooperation with Osborn Botanical Laboratory, Yale University,' New Haven, Conn.

[3] Hahn, G. G. *Phomopsis juniperovora* and closely related strains on conifers. Phytopath. **16**: 899–914. 1926.

[4] Levine, M., and H. W. Schoenlein. A compilation of culture media for the cultivation of microorganisms. 969 pp. Baltimore. 1930.

[5] Hahn, G. G., C. Hartley, and R. G. Pierce. A nursery blight of cedars. Jour. Agr. Res. [U.S.] **10**: 533–540. 1917.

ANNOUNCEMENT

The summer meeting of the Plant Pathologists of the Upper Mississippi Valley will be held August 6, 7, and 8, 1947, at the Ohio Experiment Station, Wooster, Ohio. The meeting will consist of exhibits, discussions, and a tour. A formal questionnaire has preceded this announcement. Special items to be discussed are: (1) Formal organization of a Division of The American Phytopathological Society, and (2) A plan for arranging regional projects.

H. C. YOUNG, *Chairman*
Upper Mississippi Valley Pathologists

THE RELATION OF WEATHER TO EPIPHYTOTICS OF LATE BLIGHT ON TOMATOES

A . L . H A R R I S O N

Attention was called by the writer early in 1946 (4) to an unusual outbreak of late blight of tomato, caused by *Phytophthora infestans* (Mont.) de Bary. The seriousness of the situation was stressed in that publication but the results of the outbreak proved to be far more serious than expected. The disease became extremely destructive in early tomato seed-beds over the west coast area of Florida in January. Hardly an early seed-bed escaped its ravages. From 80 to 90 per cent of the early seed-beds were complete failures and even apparently sound plants pulled from beds slightly affected with late blight were lost on being set in the field during late January.

Although late blight has been reported repeatedly as occurring on tomatoes, most of the reports have dealt with its occurrence on green fruits or on mature vines late in the season. Howitt (5) and more recently Young (10) reported its occurrence on tomato seedlings. In the outbreak of late blight in Florida, tomato seedlings growing in outdoor seed-beds were repeatedly observed to be killed by the late-blight fungus before the first true leaves were formed. In many cases whole beds involving hundreds of thousands of plants were completely destroyed before they were 3 to 4 inches high. No record in the literature has been found where late blight ever appeared so suddenly and so destructively on tomatoes over such a wide area. Before the 1946 spring season was over, it appeared in practically every tomato section in the state of Florida. It was observed first in Hillsborough County on the west coast of Florida on November 26, 1945. There were no commercial acreages of potatoes at the time in this section. It did not cause any appreciable damage to the fall crop except in isolated cases, since it did not become general over the Hillsborough-Manatee County tomato belt until late in December when the fall harvest was practically complete. There was no general killing frost to destroy the vines from the fall crop, so when the seed-beds for the spring crop were started in December and January there was plenty of inoculum to account for the disastrous outbreak of late blight in these tomato seed-beds.

The identity of the fungus responsible for the outbreak has received some attention. Some suggestions were made that it was not *Phytophthora infestans* but some other species such as *P. capsici* Leon. This would seem not to be the case since volunteer pepper plants thrived without any leaf or stem lesions in unsprayed areas of a tomato seed-bed, where tomato plants were completely destroyed. Other pepper plants without any leaf or stem lesions and adjacent to affected tomato plants were observed repeatedly. This is offered as proof that the fungus responsible for the outbreak of late blight was not *P. capsici*.

The first symptoms of late blight on young tomato seedlings appeared as small dark necrotic lesions from 4 to 5 days after periods favorable for inoculation. Sometimes they were on the leaves, sometimes on the stems, and sometimes on the growing points. The lesions rapidly enlarged and soon caused the death of the seedling. If the lesions were on the stem near the ground damping-off occurred, but usually the first lesions were on the leaves or upper portion of the stem. Young seedlings were sometimes killed within 2 or 3 days after the first symptoms were evident. Older plants took longer to succumb to the disease. Large transplants occasionally recovered from late blight when the disease had not progressed far enough to kill all the buds. Such recovery occurred only if the weather changed and became unfavorable for development of the disease. Toughened stem tissues appeared to be more resistant to invasion by *Phytophthora infestans* than were leaf and succulent stem tissues. Stem lesions on young plants were more numerous near the growing tip than they were near the surface of the ground.

A few growers who followed spray recommendations immediately after late blight first appeared in the seed-beds managed to save most of their early plants, but those growers who were caught without proper spray equipment or failed to do a thorough job of spraying lost practically all their early plants.

INFLUENCE OF WEATHER ON THE SPREAD OF LATE BLIGHT

Following the sudden outbreak of late blight in the tomato seed-beds during the middle of January the weather changed and became unfavorable for the development of late blight and favorable for the growth of tomatoes. Late tomato seed-beds came through in fine shape and the bulk of the spring tomato acreage on the west coast was set with plants from these beds.

Late blight was inactive during February but it reappeared when the weather again became favorable for the development of *Phytophthora infestans* on February 27, and continued to be favorable through March 2. From early in the morning of February 27 until the afternoon of March 2, the relative humidity did not go below 100 per cent except for about seven hours when it ranged from 95 to 100 per cent (Fig. 1). This long duration of high humidity coupled with the fact that the temperatures remained in the 60's or low 70's for the entire time made the conditions ideal for the spread and development of the late blight fungus. New lesions of late blight started to appear on March 2 and by March 4 it was destructive in most unprotected fields of tomatoes. The outbreak of late blight then subsided so that fresh lesions were hard to find by March 10. During the middle of March the weather again became favorable for the spread of late blight, which resulted in another outbreak of late blight about March 19. This phenomenon of late blight becoming destructive and then dying down was repeated six times during the 1946 spring season. There were two periods in January, three in March (the first one of these starting in February), and one in April when late blight was unusually active and destructive.

The outbreak in April was during the height of the harvest season and for this reason probably caused more financial loss than any of the other outbreaks. The farmer's auction market in Palmetto was closed on April
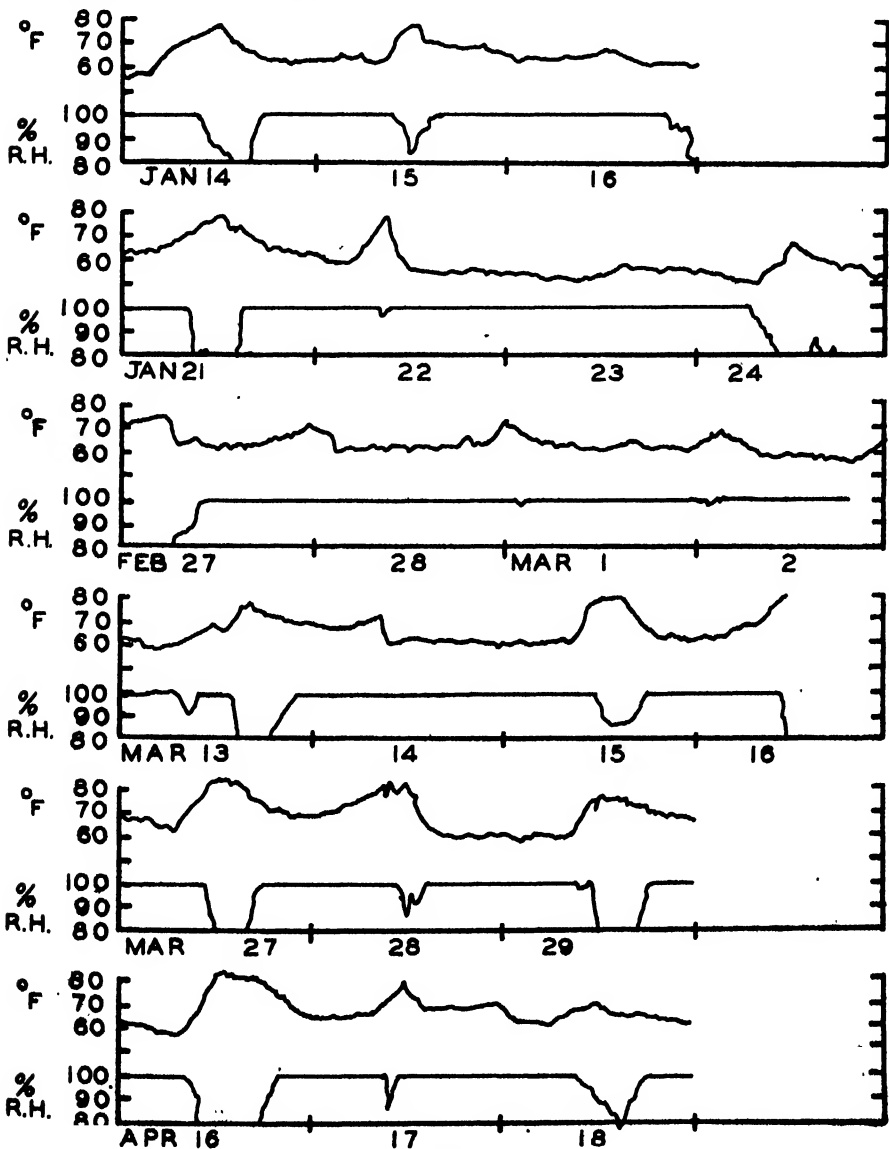
FIG. 1. Temperatures and humidities preceding late blight epiphytotics.

25 and 26 because the tomato fruits were being lost in transit from late blight. Fruits that were apparently sound began to develop late blight 4 to 5 days after they had been picked. The effect of this late spread of late blight was evident in lower prices and slower sales for several days

TABLE 1.—*Relative humidity preceding late blight epiphytotics on tomatoes in the winter of 1946*

Period	Hours the relative humidity was			
	100 per cent	90–99 per cent	80–89 per cent	Less than 80 per cent
Jan. 14, P.M.–Jan. 16, P.M.	43	4	1	0
Jan. 21, P.M.–Jan. 24, A.M.	63	2	0	0
Feb. 27, A.M.–Mar. 2, P.M.	68	7	0	0
Mar. 13, A.M.–Mar. 16, A.M.	54	3	3	0
Mar. 27, P.M.–Mar. 29, A.M.	33	2	3	0
Apr. 16, P.M.–Apr. 18, A.M.	35	1	1	0

before the market closed and continued for several days after it reopened. Growers who had kept their tomatoes sprayed as recommended had little or no loss from late blight on their fruits.

Because there were certain definite periods when late blight was destructive an attempt was made to correlate the weather with destructive outbreaks. Data on the weather that preceded the 6 serious outbreaks of late blight are presented in tables 1 and 2 and in figure 1.

In all cases late blight did not reappear in destructive form until 3 to 5 days after the beginning of these 6 periods. Long periods of high humidity were necessary for the rapid spread and development of late blight. This point is further illustrated by the fact that even though the relative humidity was 100 per cent for 10 to 15 hours practically every night during the

TABLE 2.—*Maximum and minimum temperatures and rainfall preceding late blight epiphytotics on tomatoes in the winter of 1946*

Period	Temperature, degrees F.		Rainfall, inches
	Maximum	Minimum	
Jan. 14	80.0	53.9	
Jan. 15	80.0	55.6	
Jan. 16	69.3	66.3	0.53
Jan. 21	79.8	62.0	
Jan. 22	80.4	58.8	
Jan. 23	56.2	51.2	0.73
Feb. 27	73.4	63.2	0.03
Feb. 28	74.8	62.2	3.19
Mar. 1	67.8	61.0	
Mar. 2	69.6	57.1	
Mar. 13	69.1	60.0	
Mar. 14	65.2	59.5	1.10
Mar. 15	82.9	59.2	0.26
Mar. 27	85.3	62.1	
Mar. 28	77.4	58.4	0.29
Mar. 29	78.5	65.6	0.04
Apr. 16	70.8	57.0	
Apr. 17	80.8	65.2	
Apr. 18	71.0	61.5	0.18

entire season, late blight was relatively inactive except during the 6 periods mentioned. The reason for this quiescence of late blight during most of the season is probably due to the fact that the relative humidity usually dropped to 30 to 60 per cent for several hours every day. Crosier (2) and Crosier and Reddick (3) both have demonstrated that conidia of *Phytophthora infestans* rapidly lose their viability in atmosphere with the relative humidity below 95 per cent.

Climate and other factors in relation to the spread and development of late blight have been discussed by Bonde and Schultz (1), Crosier (2), Crosier and Reddick (3), Stakman and Christensen (7), and Thomas (9), but only insofar as they are related to the disease on potatoes. In keeping with these observations on potatoes, Kern and Orton (6) and Taubenhaus and Ezekiel (8) report that periods of low temperatures and frequent rainfall are necessary for late blight to be active and destructive on tomatoes.

The observations during the present epiphytotic clearly demonstrate that *Phytophthora infestans* may become active and destructive on tomatoes with temperatures of 60° to 70° F., provided there are long periods of 100 per cent relative humidity. The weather from January 21 to 24 and from February 27 to March 2 was particularly favorable for late blight since there were 65 and 75 hours respectively of continuous high humidity with moderately low temperatures. It was following these 2 periods that most of the damage was done to the plants in the seed beds and the young plants in the field.

METHODS OF DISSEMINATION

Observational evidence during these epiphytotics clearly demonstrated that the spores of *Phytophthora infestans* might be borne for miles by the wind, provided the weather conditions were favorable. Isolated seed-beds and fields of tomatoes 30 and 40 miles from other tomatoes or potatoes were found to have late blight following some of these periods. The only possible way the fungus could have been carried to some of these fields was by the wind. The exact distances that the spores were carried or could be carried were not determined. They must have been carried 30 to 40 miles in some instances, especially to some of the fields in Collier County in south Florida. Some of the tomato fields in this county were planted miles from any habitation or other cultivated fields, yet late blight was found on tomatoes in some of these isolated fields in late January. The distance that the spores might be carried probably would depend on wind velocity, humidity, and temperature.

SUMMARY

An unusual outbreak of late blight, *Phytophthora infestans*, is reported on tomatoes from the west coast of Florida. It started on the fall crop and became destructive in the winter seed-beds and continued throughout the spring season. There were 6 definite periods when the late-blight fungus

was particularly active.   Rainfall was not so important in the spread and
development of late blight as were long periods of high humidity. Late
blight was relatively inactive unless the relative humidity was 100 per cent
for more than 15 hours.   Observational evidence indicates that spores of
Phytophthora infestans were carried by winds 30 or more miles during
certain periods when the weather was favorable.

VEGETABLE CROPS LABORATORY,
    FLORIDA AGRICULTURAL EXPERIMENT STATIONS,
      BRADENTON, FLORIDA.

LITERATURE CITED

1. BONDE, REINER, and E. S. SCHULTZ. Potato refuse piles as a factor in the dissemina-
   tion of late blight. Maine Agr. Expt. Sta. Bul. 416: 225–246. 1943.
2. CROSIER, WILLARD. Studies in the biology of *Phytophthora infestans* (Mont.) de
   Bary. N. Y. (Cornell) Agr. Expt. Sta. Mem. 155: 1–40. 1934.
3. ——— ———, and DONALD REDDICK. Some ecological relations of the potato and
   its chief fungus parasite *Phytophthora infestans*. Amer. Potato Jour. 12: 205–
   219. 1935.
4. HARRISON, A. L. Potato late blight on tomatoes on the West Coast of Florida.
   U. S. Dept. Agr., Pl. Dis. Reptr. 30: 49–50. 1946.
5. HOWITT, J. E. *Phytophthora infestans* causing a damping-off of tomatoes. Phyto-
   path. 7: 319. 1917.
6. KERN, F. D., and C. R. ORTON. *Phytophthora infestans* on tomatoes. Phytopath.
   6: 284–287. 1916.
7. STAKMAN, E. C., and C. M. CHRISTENSEN. Aerobiology in relation to plant disease.
   Bot. Rev. 12: 205–253. 1946.
8. TAUBENHAUS, J. J., and W. N. EZEKIEL. Late blight of tomatoes and potatoes.
   Tex. Agr. Expt. Sta. Circ. 60. 1931.
9. THOMAS, W. D. (JR.). Two aids for the study of potato-late-blight epidemiology.
   Phytopath. 36: 322–324. 1946.
10. YOUNG, P. A. Late blight of tomatoes in East Texas at transplanting time. Phy-
   topath. 36: 389–391. 1946.

BREEDING POTATOES FOR RESISTANCE TO RING ROT

Reiner Bonde,[1] F. J. Stevenson,[2] and
Robert V. Akeley[3]

INTRODUCTION

The ring-rot disease caused by *Corynebacterium sepedonicum* (Spieck. and Kott.) Skap. and Burk. continues to be of major importance to the potato industry of the United States and Canada (7). Baribeau in 1931 (1) reported the disease as being prevalent in potato fields in the Province of Quebec and it was discovered the following year in the United States in a field of potatoes located in Aroostook County, Maine (2). Ring rot has spread rapidly and now is present in most of the potato growing areas of the United States and in some instances has caused very large losses (8).

Progress has been made in reducing the prevalence of bacterial ring rot in Maine and elsewhere. Most of the severely infested seed stocks in Maine have been discarded.[4] However, it still is prevalent and many seed stocks still have ring rot in trace amounts. It has been very difficult to eliminate traces of the disease from slightly infected seed stocks and often they have been responsible for very rapid spread of the disease. Furthermore, seed stocks with even a trace of ring rot are not acceptable as certified seed potatoes and a trace often causes an otherwise certifiable crop to be sold at greatly reduced prices.[5] Slightly infected seed stocks are a menace wherever susceptible varieties are grown.

The best solution to the ring-rot problem is to develop highly resistant or immune varieties. Work on the development of resistant varieties has been conducted for a number of years and the progress made is summarized in this paper.

PRELIMINARY STUDIES

A search was started in 1939 to find varieties that possess resistance to ring-rot infection and that can serve as possible parents for use in a breeding program. The results of this preliminary investigation have been summarized by Bonde *et al.* (6).

[1] Plant Pathologist, Maine Agricultural Experiment Station, Orono, Me.
[2] Principal Geneticist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.
[3] Horticulturist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.
[4] Fields with 20 to 50 per cent infected plants were not uncommon in Maine in 1937 and 1938. Some growers discarded from 10 to 30 barrels of infected potatoes per acre and in some cases entire bins were badly decayed because of the prevalence of bacterial ring rot.
[5] The premium received for certified seed stocks over other seed stocks or table potatoes may be one dollar or more per hundred-weight. It is thus seen that the financial loss sustained by a farmer when a seed stock is rejected because of having a trace of ring rot may be very large.

For this work, 54 named domestic and foreign varieties and a number of unnamed seedling varieties from a wide range of sources were tested for resistance to infection by the ring-rot organism when heavily inoculated by artificial means. All but two of the named varieties were susceptible. The two resistant varieties were Friso and President (Paul Kruger), both from Holland. Two unnamed seedling selections included in this test also were highly resistant. One was from the progeny of a cross 41956 × Earlaine and the other from a cross Earlaine × 43055. The latter (46952) in addition to being resistant to ring rot is early in maturity, has attractive tubers, and is resistant to virus A in the field. Furthermore, it is self-fertile and will cross readily with other desirable varieties and selections.

Approximately 290 commercially desirable and selected seedling varieties of miscellaneous origin were tested for resistance to ring rot in other preliminary experiments. Seedling O55 (from progeny of Chippewa × Katahdin), 336–144 (from progeny of President × Katahdin), and 47102 (45146 × Earlaine) were the only ones in this experiment that failed to contract the disease.

MATERIALS AND METHODS

In view of the above findings it seemed possible to produce desirable marketing varieties of potatoes that are resistant to ring rot and to other diseases.

The highly resistant varieties referred to previously were used in making crosses, and the progenies were tested for resistance to ring rot. In some cases only one resistant parent was used and in others both parents were resistant. Resistant varieties were crossed with varieties that are resistant to late blight and to the virus diseases for the purpose of combining these desirable qualities in a single variety or potential parent. The progenies of certain selfed lines also were tested, in the hope of developing new and better parents with increased resistance. The progenies from a number of miscellaneous crosses were included so that new sources of resistance and desirable parents might be found.

The use of pure cultures of the ring-rot organism for inoculations has not given reliable results in Maine. Inoculations with hypodermic needles and by some other methods likewise have been unsatisfactory. Therefore a rather simple method was employed for the inoculations included in these studies. The diseased portions of tubers infected with ring rot were thoroughly macerated and varying amounts of sterilized rain water were added to the infective material until a thick slurry resulted.[6] The tubers of the varieties under test were cut into seed pieces of appropriate size and dipped immediately into the water suspension of the bacteria. The inoculated seed pieces were planted immediately in the field.

[6] Infected stocks of the Katahdin variety were propagated for use as inoculum for these studies. Infected tubers of this variety disintegrate to a less extent than those of some of the other commercial varieties and have served as a good source of infective material for this kind of study.

The plants that emerged from the inoculated seed pieces were examined for foliage symptoms of ring rot during the growing season and were discarded if foliage symptoms appeared. The tubers from plants that developed no foliage symptoms of ring rot were dug after frost had killed the vines and the individual tubers were clipped at the stem end and carefully observed for signs of the disease. Those tubers not showing symptoms at harvest were put in storage and examined again prior to being planted the following spring.

RESULTS

General Reaction to Ring Rot, and Other Characters, of Parents Used for Hybridizing

The studies had indicated that a few potato varieties and seedlings are very resistant to ring rot. A study to test these for possible immunity was made during the four-year period from 1942 to 1945. The results have been summarized in table 1.

TABLE 1.—*Comparison of certain commercial and seedling potato varieties with respect to susceptibility to ring rot*[a]

| Variety | Parentage | Percentage of plants infected[b] | | | | Severity of decay in tubers |
		1942	1943	1944	1945	
Green Mountain	Excelsior × Dunmore	82	90	95	80	Severe; tubers disintegrated
Houma	Chas. Downing × Katahdin	100	84	95	89	Do
Katahdin	40568 × 24642	80	65	72	68	Severe; many tubers intact and firm
Sebago	Chippewa × Katahdin	....	35	51	42	Do
President[c]	Richter's Imperator × Wilhelm Korn	0	0	2[d]	1[d]	Very slight; confined to vascular ring
47102[c] (Teton)	45146 × Earlaine	0	0	18[d]	3[d]	Do
46952[c]	Earlaine × 43055	2[d]	3[d]	2[d]	3[d]	Slight
O55	Chippewa × Katahdin	5[d]	4[d]	.....	4[d]	Do
870[c]	Earlaine × 336–144	0	0	1[d]	0	Only slight softening in vascular ring in some tubers
824[c]	Houma × 336–144	0	0	4[d]	0	Do

[a] The freshly cut surfaces of seed pieces were inoculated by being dipped in a heavy water suspension of the bacteria and planted immediately in the field.

[b] Percentages based on 10 replicated lots of 10 seed pieces each for the different varieties.

[c] Same seed stock used for this study for four successive years.

[d] Ring rot suspected, but symptoms indeterminate. Slight infection in tubers after storage.

The Green Mountain, Houma, and Katahdin varieties were very susceptible to infection and developed high percentages of the disease for all of the four years. The Katahdin variety has appeared to be somewhat less susceptible to the disease than is the Green Mountain. However, in the field, the Katahdin variety often has the disease in rather large amounts.

This might be due to the fact that it is relatively resistant to the virus diseases and, therefore, is grown for longer periods of time by farmers before the seed stock is changed, thus allowing more time for the ring-rot disease to increase.

The Sebago variety, although here classified as susceptible, appears to possess some resistance to ring rot. This seems to be confirmed by results obtained by farmers, since surveys in Maine have shown that the Sebago

TABLE 2.—*Reaction to ring rot and desirable characteristics of named and seedling potato varieties used as parents*

Variety	Reaction to ring rot [a]	Superior characters	
		Disease resistance	Other characters
Chippewa	S	Mild mosaic virus A	Tuber shape, yield
Earlaine	S		Pollen fertility, earliness, tuber shape
Earlaine No. 2	S		Yield
Friso	R		
Green Mountain	S		Yield, cooking quality
Houma	S		Tuber shape, cooking quality
Katahdin	S	Mild mosaic virus A	Pollen fertility, tuber shape
Ostragis	S	Scab	
President	R	Late blight	
Sebago	S	Late blight, scab	
Sequoia	S	Insects	Cooking quality
O55	R	Southern brown rot	
70–7	S	Late blight	
76–49	S	Late blight	
96–28	S	Late blight	
96–44	S	Late blight	
96–56	S	Late blight	
96–345	S	Late blight	
336–18	R	Late blight	
336–123	S	Late blight	
336–144	R	Late blight	
447–4	S		
918–9	S	Late blight	
918–12	S	Late blight	
3895–3	S	Late blight	Earliness
3895–13	S	Late blight	Earliness
46952	R		Pollen fertility, earliness, tuber shape
47102 (Teton)	R	Virus X	Pollen fertility, tuber shape

[a] Reactions were susceptible (S), or resistant (R) in tests for three or more successive years with inoculated seed stocks planted in the field.

seed stocks are relatively free of ring rot. Also, the symptoms of the disease are slow to manifest themselves in inoculated plants, and this too would indicate that this variety is somewhat resistant.

The President variety, as well as seedlings 47102 (recently named Teton), 46952, O55, 870, and 824 are resistant to ring rot and showed no infection, or very little, in the field (Table 1). These varieties, however, are not immune. Careful examination of the tubers from inoculated plants showed that the above-mentioned resistant varieties may become infected to a small degree with the symptoms in plants and tubers not very obvious. Seedlings

47102 and 46952 are self-fertile and now are used extensively in the breeding program for ring-rot resistance. President and O55 also have been used as parents. Seedlings 870 and 824 are probably the most resistant selections that have been made in Maine. The latter produced viable pollen in the greenhouse at Beltsville, Maryland, in 1946. Selfed seed was obtained and crosses made in which 824 was used as a pollen parent.

Twenty-eight parents were used in producing the progenies that were studied with regard to their resistance to ring rot. Table 2 summarizes the reactions of these parent varieties to ring-rot infection and gives some of their desirable characters. It may be noted from the data in table 2 that the 28 parents possess a wide range of characteristics that would be desirable to combine in varieties to be grown in Maine. It is of special value that Katahdin, 46952, and 47102 produce fertile pollen and readily fertilize most of the other varieties used as parents. Friso does not readily produce seed and requires special treatment and care when used for breeding purposes.

General Reaction of Progenies to Ring Rot

Table 3 gives a summary of the reaction to ring rot of 49 different progenies, the parents of which have been listed and briefly described in table 2. An analysis of the data in table 3 shows that in the 13 progenies with both parents resistant, the percentage of resistant seedlings ranged from 53.0 to 89.9 and averaged 68.9; in the 28 progenies with one parent resistant, the range was 11.1 to 76.9 per cent and the average was 42.7 per cent; and in the 8 progenies with neither parent resistant, the range was 0 to 50.0 per cent and the average was 8.8 per cent.

It may be noted in table 2 that a number of the parents are susceptible to ring rot. The data in table 3 show that usually no resistant seedlings resulted when these parents were self-fertilized or were cross-fertilized with other susceptible varieties. The progenies listed in table 3 that produced no resistant seedlings are those from Chippewa selfed, Sebago × Ostragis, Sequoia × 96–56, 3895–3 × 447–4, 3895–13 × Earlaine, and 336–123 × 47156. Sebago appears to be somewhat resistant to ring rot, and a few resistant seedlings (20 per cent) resulted when it was hybridized with the susceptible variety, Earlaine. The progeny of 96–56 × Katahdin gave more resistant seedlings (50 per cent). Thus there is evidence that some susceptible varieties possess factors for ring-rot resistance that are transmitted to the progeny.

The seven ring-rot resistant varieties given in table 2 were hybridized with the different susceptible varieties that are listed. Twenty-eight progenies were obtained in which a susceptible and a resistant parent were used in making the crosses.

Resistance to ring rot was inherited and was transmitted to the progenies when one of the parents was resistant. The percentage of resistant seedling varieties that resulted from crossing a resistant parent with a susceptible one varied from 11.1 to 76.9 per cent, with an average of 42.7 per cent.

TABLE 3.—*General reactions of different progenies and Katahdin controls to ring rot, 1942 to 1945*

Variety or cross and reaction to ring rot[a]	Seedlings or lots tested[b]	
	Number	Percentage resistant[c]
Katahdin (control)	520	0.0
Chippewa (S), Selfed	110	0.0
Chippewa (S) × 336–144 (R)	120	40.2
Chippewa (S) × 47102 (R)	79	44.7
Earlaine (S) × 336–144 (R)	90	32.9
Earlaine (S) × 46952 (R)	132	45.5
Earlaine No. 2 (S) × 46952 (R) . ..	9	11.1
Friso (R) × Katahdin (S)	120	45.8
Green Mountain (S) × 336–144 (R)	84	46.4
Green Mountain (S) × 46952 (R)	17	30.6
Houma (S) × 336–144 (R)	128	46.6
President (R), Selfed	127	54.4
President (R) × Chippewa (S)	18	40.0
President (R) × Earlaine (S)	353	38.2
President (R) × Katahdin (S)	336	43.2
President (R) × 76–7 (S)	15	40.0
President (R) × 76–49 (S)	95	43.2
President (R) × 96–28 (S)	214	43.6
President (R) × 96–56 (S)	89	44.9
President (R) × 336–144 (R)	140	74.7
President (R) × 918–12 (S)	80	66.0
President (R) × 46952 (R)	113	53.0
President (R) × 47102 (R)	17	64.7
President (R) × 47156 (S)	385	37.8
Sebago (S) × Ostragis (S)	18	0.0
Sebago (S) × Earlaine (S)	97	20.0
Sebago (S) × 336–144 (R)	15	. 47.0
Sebago (S) × 46952 (R)	112	13.0
Sequoia (S) × 96–56 (S)	12	0.0
O55 (R) × 47102 (R)	28	71.4
96–28 (S) × 336–144 (R)	138	41.2
96–44 (S) × 336–144 (R)	165	54.5
96–56 (S) × 336–144 (R)	162	40.7
96–56 (S) × Katahdin (S)	4	50.0
96–345 (S) × 336–144 (R)	187	47.8
336–18 (R), Selfed	103	81.4
336–18 (R) × 96–56 (S)	217	52.5
336–18 (R) × 46952 (R)	12	58.3
336–123 (S) × 47156 (S)	20	0.0
336–144 (R), Selfed	60	71.0
918–9 (S) × 46952 (R)	68	44.0
3895–3 (S) × 447–4 (S)	94	0.0
3895–13 (S) × Earlaine (S)	22	0.0
46952 (R), Selfed	39	66.2
46952 (R) × 336–144 (R)	184	53.7
46952 (R) × 47102 (R)	79	89.9
46952 (R) × Katahdin (S)	5	40.0
47102 (Teton) (R), Selfed	149	85.9
47102 (R) × 96–56 (S)	195	76.9
47102 (R) × 336–144 (R)	25	72.0

[a] (S) indicates that parent is susceptible and (R) that parent is resistant.
[b] From 5 to 10 freshly cut seed pieces of each seedling or of each Katahdin lot were inoculated by being dipped in a heavy suspension of the bacteria and were then planted immediately in the field.
[c] Those classed as resistant showed no symptoms of ring rot in foliage or tubers.

The variety President was one of the first that was found in Maine to be resistant to ring rot and it has been used extensively for producing some of the progenies used in these studies. Approximately 1725 seedlings secured by crossing President and a susceptible variety were tested for resistance to ring rot. Of these 773, or 44.8 per cent, were resistant.

Seedlings 336-18 and 336-144 which were secured from the progeny of President × Katahdin are resistant to ring rot and late blight and have produced good yields in Maine. Two hundred seventeen seedlings, having as parents 336-18 and the ring-rot-susceptible seedling variety 96-56, were included in the test, of which 114, or 52.5 per cent, were resistant. The resistance in 336-144 also was transmitted. There were 1089 seedlings tested that had 336-144 and some susceptible variety as parents, of which 489, or 44.9 per cent, failed to contract ring rot. It is of interest here that 336-123, which also has President and Katahdin as parents, is resistant to late blight and is phenotypically similar to 336-18 and 336-144 but is very susceptible to ring rot. No ring-rot-resistant seedlings were obtained in the progeny when 336-123 was hybridized with susceptible 47156.

Friso and 47102 also produce progenies with a high percentage of resistant seedlings when they are used for making crosses (Table 3). Friso crossed with the susceptible Katahdin gave 120 seedlings of which 55, or 45.8 per cent, were resistant to ring rot. When 47102 was crossed with the susceptible Chippewa variety, 44.7 per cent were resistant and when it was crossed with 96-56, 76.9 per cent were resistant.

Seedling 46952, although a desirable parent for breeding purposes, has not been considered as resistant to ring rot as some of the other selections that were used. Of 343 seedlings tested that had 46952 and some susceptible variety as parents, 105, or 30.6 per cent, possessed resistance to the disease.

The data also show that resistance to ring rot is accumulative and can be increased by combining the factors from different parents. When the resistant parents President, 336-18, 336-144, 46952, and 47102 were self-fertilized, from 54.4 to 85.0 per cent of the seedlings in the progenies were resistant. Likewise, when both of the parents were resistant, from 53 to 89.9 per cent of the seedlings were resistant.

The Percentage of Inoculated Seed Pieces Producing Plants with Symptoms

The ring-rot infection that occurred in the parents and progenies of 33 crosses tested in 1944 and 1945 is given in table 4. The extent of ring-rot infection is shown in table 4 in five classes based on the percentage of plants in each lot that contracted the disease.

The lots in Class 0 developed no infection as a result of the inoculations and are considered as being highly resistant. Those in Class 1 had some degree of resistance inasmuch as only one or two plants out of ten became infected and the degree of infection in the tubers also was slight. The lots

TABLE 4.—*Percentage and severity of infection in plants from inoculated seed pieces of Katahdin controls, parents of crosses, and their progenies, in 1944 and 1945*[a]

Variety or cross and reaction to ring rot[b]	Lots tested	Percentage of lots in each infection class[c,d]						Lots infected
		Class 0	Class 1	Class 2	Class 3	Class 4	Class 5	
Controls and parent lots	*No.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Per cent*
Katahdin controls	500	....	. .	4	6	50	40	100
Chippewa (S)	20	. ...	.		..	20	80	100
Earlaine (S)	20	.. ..	....	.	. ..	......	100	100
Earlaine No. 2 (S)	20	. ...	.	.	. .	..	100	100
Green Mountain (S)	20	.....	.	...	...	20	80	100
Houma (S)	20	..	..	. .	. ...	..	100	100
President (R)	20	100			..	. ..		0
Sebago (S)	20	..... .	10	20	20	30	20	100
Sequoia (S)	20	. . .		20	60	20	....	100
O55 (R)	10	90	10	. .	.	...	.. .	10
B76-7 (S)	10	...	10	20	40	10	20	100
B76-49 (S)	20	.. ..	10	20	50	20	0	100
96-28 (S)	20	...	....	.	. .	10	90	100
96-44 (S)	20	.....	.	20	. .	10	70	100
96-56 (S)	20	. .	.	. .	. .	.....	100	100
96-345 (S)	20	. .	.....	... .	......	20	80	100
336-18 (R)	10	100	. ..	.	....	......	. . ..	0
336-144 (R)	20	100	. .	....	....	... .	. . ..	0
46952 (R)	20	90	10	...	....	......	. ..	10
47102 (Teton) (R)	50	100	. ...	......	....	. ..	....	0
47156 (S)	10	. ..	......	. ..	...	.. ..	100	100
Progeny lots								
Chippewa (S) × 47102 (R)	79	75	11	5	4	3	3	26
Earlaine (S) × 336-144 (R)	56	47	14	11	7	2	20	54
Earlaine (S) × 46952 (R)	49	53	0	12	10	4	20	46
Earlaine No. 2 (S) × 46952 (R)	9	11	0	11	22	11	45	89
Green Mountain (S) × 336-144 (R)	84	45	5	5	4	1	41	56
Green Mountain (S) × 46952 (R)	17	71	12	0	12	0	6	30
Houma (S) × 336-144 (R)	69	54	3	1	3	3	36	46
Sebago (S) × 46952 (R)	57	5	4	5	7	4	76	96
Sequoia (S) × 96-56 (S)	6	0	17	0	17	0	66	100
President (R) × Self	113	55	13	11	5	6	10	45
President (R) × Katahdin (S)	106	31	7	17	7	2	37	70
President (R) × B76-7 (S)	15	40	27	13	7	0	13	60
President (R) × B76-49 (S)	95	43	21	7	7	2	19	56
President (R) × 96-28 (S)	215	43	7	10	7	6	28	58
President (R) × 96-46 (S)	89	45	17	7	8	9	15	56
President (R) × 47102 (Teton) (R)	17	65	18	6	6	0	6 .	36
President (R) × 47156 (S)	108	55	4	7	1	2	31	45
O55 (R) × 47102 (R)	28	71	14	4	4	4	4	30
96-28 (S) × 336-144 (R)	138	41	12	7	8	6	26	59
96-44 (S) × 336-144 (R)	165	55	7	3	7	3	26	46
96-56 (S) × 336-144 (R)	64	31	2	5	8	13	42	70
96-345 (S) × 336-144 (R)	107	56	12	1	3	5	23	44
336-18 (R) × Self	77	82	16	1	1	0	0	18
336-18 (R) × 96-56 (S)	205	47	17	10	12	4	10	53
336-18 (R) × 46952 (R)	12	58	17	17	8	0	0	42
336-144 (R) × Self	24	79	0	0	4	8	8	20
46952 (R) × Self	22	77	9	9	0	0	5	23
46952 (R) × 336-144 (R)	42	54	2	3	4	4	32	45
46952 (R) × 47102 (R)	71	92	6	0	0	0	2	8
46952 (R) × Katahdin (S)	5	20	40	0	0	0	40	80

TABLE 4.—(*Continued*)

| Variety or cross and reaction to ring rot[b] | Lots tested | Percentage of lots in each infection class[c, d] | | | | | | Lots infected |
		Class 0	Class 1	Class 2	Class 3	Class 4	Class 5	
Progeny lots (Continued)	*No.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Per cent*
47102 (R) × Self	149	85	8	2	1	1	4	16
47102 (R) × 96–56 (S)	195	77	8	6	4	3	2	23
47102 (R) × 336–144 (R)	25	72	24	0	0	0	4	28

[a] Ten freshly cut seed pieces of each variety or lot were inoculated by being dipped in a heavy suspension of the bacteria and were planted immediately in the field.

[b] (S) indicates that parent is susceptible and (R) that parent is resistant.

[c] Class of infection   Plants infected
    0   None
    1   1–2   slightly infected
    2   3–4   severely infected
    3   5–6   severely infected
    4   7–8   severely infected
    5   9–10 severely infected.

[d] Total in all classes will not always be 100 per cent, due to use of round numbers.

listed in Classes 2, 3, 4, and 5 are considered definitely susceptible, 3 to 10 plants out of 10 becoming infected and the severity of infection as measured by the amount of rot in the tubers also being great.

Katahdin controls and the parents Chippewa, Earlaine, Earlaine No. 2, Green Mountain, Houma, Sequoia, 96–28, 96–44, 96–56, 96–345, and 47156 are all definitely susceptible to ring rot, with no lots in Classes 0 and 1 (Table 4). Sebago, B76–7, and B76–49 possessed some resistance as is shown by the fact that some lots were in Class 1. In President, 336–18, 336–144, and 47102 all of the lots were in Class 0, which indicates considerable resistance. Seedlings O55 and 46952 were somewhat less resistant, some lots being in Class 1.

The data in table 4 confirm those presented in table 3 and show that a high percentage of resistant seedlings occur in the progenies when one or both of the parents are resistant to the disease. From 5 to 77 per cent (average 45) of the lots in the different progenies were in the resistant Class 0 when one parent was resistant, and from 54 to 92 per cent (average 71.8) were in Class 0 when both parents were resistant, including cases where the resistant parents were self-fertilized. When President, 336–18, 336–144, 46952, and 47102 were selfed, 55, 82, 79, 77, and 85 per cent, respectively, of the seedlings in the progenies were resistant and in Class 0. Furthermore, an additional number was in Class 1 and showed only a slight degree of infection.

Reliability of Ring-Rot Inoculation of Seedlings Selected as Being Resistant

The question naturally arises whether the seedlings classified as resistant in tables 3 and 4 are actually resistant to ring rot or whether they merely escaped infection when artificially inoculated.

Some data were secured that pertain to this problem. Three hundred forty-seven seedlings that were selected as resistant in 1944 were reinoculated in 1945 and observations made on the development of the disease.

It was observed that 286 or 82.42 per cent of the 347 seedlings found to be free of ring rot in 1944 continued to be free of infection after inoculations in 1945. There were very few actual "escapes." Most of the seedlings that became infected in 1945 contracted the disease in relatively few plants or tubers. Furthermore, in many cases the degree of infection was slight and the bacteria were localized in relatively small areas of the tubers. The disease also was difficult to detect in some seedlings, because only very slight symptoms were apparent.

The writers believe that the method used for testing resistance to ring rot is reliable. Most of the highly susceptible seedlings are readily detected and those that survive the test have varying degrees of resistance.

Severity of Infection in Tubers in Progenies from Crosses Including Resistant Varieties

Studies described above have shown that the progenies resulting from crossing certain ring-rot-resistant and susceptible varieties contain high percentages of new seedling varieties that are resistant to the disease. The results were based on the presence or absence of foliage and tuber symptoms of the disease, no attention being paid to the extent and severity of the tuber rot that resulted.

An experiment was conducted to ascertain if resistance to ring rot in the tubers, based on percentage of tubers infected and severity of decay, also is transmitted to the progenies when resistant parents are used for making crosses.

Tubers from inoculated plants of 410 resistant seedlings selected from 12 crosses were put into storage and carefully examined for the presence of ring rot the following spring. The seedlings were graded into five classes according to the percentage of tubers infected and the severity of decay. Class 0 had no infection. In Class 1 there was a trace of infection in less than 25 per cent of the tubers. In Class 2 there was a trace of infection in 26 to 50 per cent of the tubers. In Class 3, 51 to 75 per cent of the tubers were infected and the decay was usually medium in severity. In Class 4, 76 to 100 per cent of the tubers were infected and the decay usually was very severe.

The data are summarized in table 5 and show that the tubers of the parent varieties Katahdin, Chippewa, Earlaine, Houma, 96–56, and 96–345 were included mostly in the highly susceptible Classes 3 and 4. The Sebago variety was somewhat less susceptible to ring rot and 15 per cent of the tubers showed no symptoms of the disease.

On the other hand, the parent varieties 46952, Friso, President, and 336–144 were resistant to ring rot, with from 70 to 75 per cent of the tubers disease-free and included in Class 0. From 10 to 20 per cent of the remaining tubers developed only a slight degree of rot.

Resistance to ring rot in the tubers was transmitted to the progeny when the resistant parents were used for making the crosses.

From 37 to 75 per cent of the progenies showed no tuber decay and were included in Class 0 when seedling 336–144 was used as one parent in making crosses with susceptible varieties. In addition, from 7 to 35 per cent of these progenies developed a very slight degree of infection, being in Class 1. The progenies with no tuber infection were increased to 83 per cent when the resistant parent 336–144 was crossed with the somewhat resistant parent 46952.

Tuber resistance in the varieties President, Friso, and 46952 likewise was transmitted to the progenies when these three varieties were used as parents in making the crosses (Table 5).

The reaction to tuber decay of the seedlings selected as being resistant

TABLE 5.—*Resistance to tuber infection of controls, parents, and resistant selections from different progenies. Crop of 1944*

Variety or cross and reaction to ring rot[a]	Lots tested[b]	Lots in each class of infection					Lots infected
		Class 0	Class 1	Class 2	Class 3	Class 4	
	No.	Pct.	Pct.	Pct.	Pct.	Pct.	Per cent
Controls and parent lots							
Green Mountain controls	200[d]	0	0	0	40	60	100
Katahdin (S)	20	0	0	10	30	40	80
Chippewa (S)	20	0	0	5	25	50	80
Sebago (S)	20	15	5	10	20	10	45
Earlaine (S)	20	0	0	0	20	50	70
Houma (S)	20	0	0	10	35	40	85
96–56 (S)	20	0	0	15	40	30	85
96–345 (S)	20	0	0	25	30	25	80
46952 (R)	20	70	20	0	0	0	20
Friso (R)	20	75	10	0	0	0	10
President (R)	20	70	20	0	0	0	20
336–144 (R)	20	70	10	0	0	0	10
Progeny lots							
Chippewa (S) × 336–144 (R)	46	37	35	15	11	2	63
President (R) × 336–144 (R)	63	70	24	6	0	0	30
Houma (S) × 336–144.(R)	23	74	26	0	0	0	26
96–56 (S) × 336–144 (R)	33	75	19	3	3	0	25
96–345 (S) × 336–144 (R)	29	75	7	13	4	0	24
46952 (R) × 336–144 (R)	12	83	17	0	0	0	17
Sebago (S) × Earlaine (S)	6	50	17	17	17	0	51
President (R) × Earlaine (S)	49	75	19	6	0	0	25
President (R) × Katahdin (S)	80	45	27	22	5	1	55
Sebago (S) × 46952 (R)	4	50	25	0	0	25	50
Earlaine (S) × 46952 (R)	15	67	27	7	0	0	34
Friso (R) × Katahdin (S)	50	52	28	14	6	0	48

[a] (S) indicates that parent is susceptible and (R) that parent is resistant.
[b] Five seed pieces for each lot or seedling tested were inoculated by being dipped in a heavy suspension of bacteria in water and were planted immediately in the field.
[c] Based on symptoms in tubers cut after being in storage: 0 = no infection on tubers when cut; 1 = trace infection in 1 to 25 per cent of tubers; 2 = trace infection in 26 to 50 per cent of tubers; 3 = infection in 51 to 75 per cent of tubers, with decay usually medium in severity; 4 = infection in 76 to 100 per cent of tubers, with decay usually very severe.
[d] Used tubers from 200 infected plants.

to ring rot was in striking contrast to that which developed in the Green Mountain controls. The tubers of 200 Green Mountain plants infected with ring-rot bacteria likewise were placed in storage and examined. All tubers had the disease, with a high percentage completely disintegrated when examined. All of the tubers from the infected Katahdin plants also had the disease, but the extent of the decay was less severe and many of the tubers were still relatively firm and intact.

Combination of Ring-Rot Resistance with Desirable Tuber Type and Other Marketing Qualities

The seedlings from 12 progenies were inoculated with ring-rot bacteria for two successive years, and the percentage of resistant seedlings for each year was recorded. The seedling varieties that became infected were dis-

TABLE 6.—*Proportion of ring-rot-resistant seedlings possessing desirable marketing qualities and plant characteristics in 1944 and 1945*

Parentage	Seedlings resistant to ring rot in field test[a]	Resistant seedlings with desirable marketing qualities	
	No.	No.	Per cent
Sebago × Earlaine	98	1	1.0
President × Earlaine	180	1	0.5
President × 336–144	175	3	0.6
President × Katahdin	125	4	3.2
Houma × 336–144	60	2	3.3
336–144 selfed	27	0	0.0
336–18 selfed	77	0	0.0
Friso × Katahdin	120	26	26.7
96–345 × 336–144	120	20	16.7
46952 × 47102	71	50	70.0
Chippewa × 47102 (Teton)	79	32	40.0
47102 (Teton) × 96–56	195	63	32.3
Katahdin controls	125	0	0.0

[a] Freshly cut seed pieces of each lot were inoculated with ring-rot bacteria and were planted in the field. Only those are included here that were resistant to ring rot.

carded each season, as also were those that had undesirable tuber type or plant characteristics. Table 6 summarizes the results.

All of the Katahdin controls developed ring rot when inoculated with the pathogen (Table 6). Relatively few seedlings from the progenies of Sebago × Earlaine, President × Earlaine, President × 336–144, Houma × 336–144, and President × Katahdin possessed good tuber type and also remained free from ring rot for the two seasons. It was shown in previous experiments that the President variety and the related seedlings 336–18 and 336–144 when used as parents produce progenies with a high percentage of resistant seedlings. However, many of these seedlings, although resistant to ring rot, have excessively long stolons, and are too late in maturing to be considered suitable for Maine. Moreover, many of the tubers are of poor ape or cling too tightly to the vines. Therefore President and other re-

lated varieties, including 336–18 and 336–144, are not desirable parents for the production of new varieties that will be adapted to Maine.

Fortunately, however, there are other parents that transmit ring-rot resistance, along with desirable tuber type and other marketing qualities. It may be noted in table 6 that when Friso was crossed with Katahdin, nearly 27 per cent of the progeny were resistant to ring rot and also possessed desirable plant characteristics and tuber type. Friso, however, is not self-fertile and does not readily retain its flowers when fertilized by other varieties and, therefore, is not a desirable parent. When 96–345 was crossed with 336–144, approximately 17 per cent of the seedlings were resistant and otherwise desirable. It appears that 96–345, which is resistant to late blight, is a good parent to use in combination with other seedlings that are resistant to ring rot.

Seedlings 46952 and 47102 are resistant parents with viable pollen. When these parents were used for making crosses, a high percentage of the seedlings in the progenies were not only resistant to ring rot but also possessed attractive tubers and plants with desirable cultural characteristics. When 46952 was crossed with 47102, both of which are resistant, 70 per cent of the progeny also were resistant. Furthermore, none of the seedlings in the progenies were excessively late in maturing and the tubers in many cases were attractive. When 47102 was crossed with the susceptible varieties Chippewa and 96–56, 40.0 and 32.3 per cent, respectively, of the seedlings in the progenies were resistant to ring rot and many of these had attractive tubers and were relatively early in maturity.

Comparison of Yields of Ring-Rot-Resistant Seedlings With Yields of Standard Varieties

A relatively large number of ring-rot-resistant seedlings have been developed in Maine as a result of the breeding program. Most of these are of rather recent origin and have not been studied critically regarding their performance in the field.

Data were secured in 1945 on the yields of four ring-rot-resistant seedling varieties in comparison with Pawnee, a newly named variety, and with four standard varieties now being grown in Aroostook County, Maine (Table 7).

Seedling 47102 gave the highest yield among the nine varieties tested. It also was superior in the general appearance of the crop, the tubers being uniform in size and having an attractive white color. It, however, is somewhat less smooth than some of the newer varieties that recently have been developed. This seedling has been described by Starr and Riedl as being resistant to ring rot and as possessing good cooking and keeping qualities (9, p. 14). Studies conducted in Maine confirm the results of Starr and Riedl that this variety is very resistant (3, p. 508; 4, p. 214; and 5, p. 421). It has contracted only a trace of the disease, over six successive years, even after freshly cut seed pieces were heavily contaminated with viable ring-rot bacteria prior to being planted in the field. The successive seed stocks.

grown from seed pieces of this variety inoculated with the disease organisms in 1941 and 1942 have shown no apparent symptoms even at this date. In contrast, all of the tubers and plants from inoculated seed stocks of the Katahdin and Green Mountain varieties developed symptoms of ring rot.

In one yield test Green Mountain, long considered a high yielding variety in Maine, produced 15 barrels or 41 bushels less per acre than did seedling 47102. Furthermore, 20 per cent of the Green Mountain tubers developed leafroll net necrosis, a condition that was not noted in the new seedling variety.

Seedling 336–144 has been resistant to ring rot, has produced good yields, and possesses some resistance to late blight. It, however, is too late

TABLE 7.—*Yields of four ring-rot-resistant seedling varieties compared with Pawnee and four standard varieties*

Variety or selection	Characteristics of varieties	Yields per acre[a]	
		Barrels	*Bushels*
O55	Late, resistant to ring rot, with good tuber shape	119	328
46952	Early, resistant to ring rot, with good tuber shape	114	314
336–144[b]	Very late, resistant to ring rot, somewhat resistant to late blight	142	418
47102 (Teton)	Medium late, resistant to ring rot, yielding well	178	492
Pawnee	Medium early, promising for Greeley, Colorado, and other areas	154	424
Green Mountain	Late standard variety for Maine, with good cooking quality	163	451
Katahdin	Late standard variety for Maine	116	320
Sebago	Late standard variety for Maine	137	377
Irish Cobbler	Early standard variety for Maine	137	377

[a] Based on six replicated plots of 25 hills each for each variety. Minimum required for significance is 29.1 barrels or 80.0 bushels at the 1 per cent level and 21.7 barrels or 59.9 bushels at the 5 per cent level.

[b] Seed stock had 20 per cent leaf roll.

in maturing to be grown commercially in northern Maine. It also appears to be very susceptible to leafroll.

Seedlings O55 and 46952, which are resistant to ring rot, gave rather low yields in these tests. However, 46952 possesses viable pollen and produces seedlings with attractive tubers when used as a parent in crosses with other varieties.

It may be noted that the Katahdin variety yielded only 116 barrels, or 320 bushels, per acre in these tests. This variety often has produced rather low yields in Maine in comparison with some of the older varieties.

It would seem to be possible to develop new varieties that would consistently yield more than the Katahdin, be resistant to ring rot and the other diseases, and also possess good cooking and marketing qualities.

DISCUSSION

Resistance to ring rot has been found in relatively few varieties. These varieties were obtained originally from a number of widely separated sources, namely Friso and President from the Netherlands and a number of unrelated seedling varieties produced in the United States (6). More recently, resistance has been found in a number of other unrelated selections. Since ring-rot resistance has been found in such unrelated material it would seem desirable to test in the future more varieties from different sources to make additional selections that may be used for producing varieties that are resistant to ring rot.

A number of varieties and seedlings used as parents, as well as the progenies from different crosses, were tested for resistance to ring rot when inoculated by artificial methods. The data show that no, or very few, resistant seedlings are produced when certain susceptible parents are intercrossed. However, when parents known to carry factors for resistance are crossed with susceptible varieties, a large number of resistant seedlings appear in the progenies. The relative number of resistant seedlings that are produced is increased further if the resistant selections are inbred or are crossed with other resistant varieties.

Resistance to ring rot is inherited and it is possible to produce resistant varieties with desirable marketing qualities. President, seedlings 336–144 and 336–18 (both from the cross President × Katahdin), and other related parents transmit a high percentage of resistance to the progenies. However, most of these resistant seedlings are very late, have ill-shaped tubers, and are otherwise undesirable. Because of this, earlier resistant varieties are now being used as parents for making crosses. Seedlings 46952 and 47102 transmit ring-rot resistance and also produce seedlings that possess good cooking and marketing qualities.

Furthermore, when these resistant parents are crossed with selections from U.S.D.A. crosses 76, 96, and 918, which are resistant to late blight, a large percentage of the seedlings in the progenies also is highly resistant to late blight, besides being resistant to ring rot.

Degrees of resistance and susceptibility were apparent throughout the tests. Some of the selections were very resistant and no, or very few, diseased plants or tubers resulted because of the inoculations. Other seedlings became infected but in a relatively small percentage of the plants and tubers. Some were tolerant to ring rot and developed only slight symptoms of the disease, and the tubers remained relatively firm and intact even when infected. There also were some that manifested severe symptoms in that the plants wilted and died rapidly and the tubers soon disintegrated.

According to the data in this paper, the resistant seedling varieties that have been developed are not immune from the disease. The symptoms in some cases are not distinct and the plants are rather tolerant. It may be justly asked whether such varieties will be desirable and whether they may not serve as perpetual sources of infection.

The writers believe that highly resistant varieties will be of value even if they are not immune. The results presented are based on artificial inoculations which were much more severe than the contamination that may normally occur in the field or in the farmers' storage houses. The controls and other commercial varieties were all infected by the first year's inoculations. However, inoculation of certain resistant varieties resulted in none or small percentages of infection, and a repetition of inoculation in the three succeeding years also resulted in very few infections. One resistant variety has been tested for six successive years with only a trace of ring rot resulting. The writers have concluded that some of the resistant varieties discussed here would not have become infected with ring rot under normal farm conditions. One of these (47102) has recently been named "Teton" and has been distributed to a number of growers.

SUMMARY

Ring rot, caused by *Corynebacterium sepedonicum* (Spieck. and Kott.) Skap. and Burk., continues to be a major potato disease. Many growers have been unable to secure and maintain healthy seed stocks and in many cases have suffered large losses. The availability of resistant or immune varieties would help greatly to control ring rot.

The resistant variety President and five resistant seedling selections were severely inoculated with ring-rot bacteria and tested for resistance to ring rot for four successive years. These varieties contracted very little disease as a result of the tests. In contrast, the susceptible varieties developed a high percentage of infection and none survived the test for more than one year.

Forty-nine progenies derived from crosses involving resistant and susceptible parents were tested for resistance to ring rot. No resistant seedlings, or very few, occurred in the progenies when both of the parents were susceptible. A relatively high percentage of a progeny was resistant when one or both parents were resistant. The percentage of resistant seedlings was increased when both of the parents were resistant or when resistant parents were self-fertilized.

The variety President and the related selections (336–18, 336–144, etc.) produced high percentages of ring-rot-resistant seedlings when used in the breeding program. These seedlings, however, are too late in maturing and their tubers are too ill-shaped for them to be of value as commercial varieties.

Seedlings 46952 and 47102 are resistant and have viable pollen. They produced progenies with high percentages of resistant seedlings that have good marketing quality.

Friso, a Dutch variety, also produced a high percentage of resistant seedlings with good marketing qualities but is pollen-sterile and does not readily produce viable seed.

The method used in this study for testing the seedlings for resistance to

ring rot was found to be fairly accurate. There were very few actual escapes and plants that survived the test in most cases possessed a high degree of resistance.

Resistance of the tubers to ring-rot decay also was transmitted to the progenies when resistant parents were used for making the crosses.

When the ring-rot-resistant seedlings 46952 and 47102 were crossed with certain parents that are resistant to late blight, a number of selections were secured that are highly resistant to both diseases.

Seedling 47102, which is very resistant to ring rot, gave the highest yield in 1945 in comparison with five standard varieties and three new seedling varieties. This selection has been named Teton and distributed to a number of growers.

With the desirable parent material now available, it should not be difficult to produce new varieties with good marketing qualities that also are resistant to ring rot and other diseases. It appears to be chiefly a matter of making the necessary crosses, selecting from the progenies the seedlings that possess the desirable characters, and testing them for resistance to ring rot and other diseases.

MAINE AGRICULTURAL EXPERIMENT STATION,
    ORONO, MAINE
        AND
U. S. DEPARTMENT OF AGRICULTURE.

LITERATURE CITED

1. BARIBEAU, BERNARD. Bacterial wilt of potatoes. Can. Pl. Dis. Surv. Rept. 11: 49. 1931.
2. BONDE, REINER. A bacterial wilt and soft rot of the potato in Maine. Phytopath. 27: 106–108. 1937.
3. ——————, M. R. HARRIS, and DONALD MERRIAM. Ring-rot resistance in progenies from crosses with resistant varieties. Me. Agr. Exp. Sta. Bul. 438. 1945.
4. ——————, and STANISLAS SNIESZKO. Ring-rot resistance in progenies from crosses with resistant varieties. Me. Agr. Exp. Sta. Bul. 426. 1944.
5. ——————, and ——————. Varieties resistant to ring rot. Me. Agr. Exp. Sta. Bul. 420. 1943.
6. ——————, F. J. STEVENSON, C. F. CLARK, and ROBERT V. AKELEY. Resistance of certain potato varieties and seedling progenies to ring rot. Phytopath. 32: 813–819. 1942.
7. KOEHNKE, MARX. Prevalence of ring rot in certified seed-producing areas in the United States and Canada. Amer. Pot. Jour. 22: 21–27. 1945.
8. LEACH, J. G. Report of the committee to coordinate research on new and unusual potato diseases. Amer. Pot. Jour. 17: 81–88. 1940.
9. STARR, G. H., and W. A. RIEDL. Potato ring rot and its control. Wyoming Agr. Exp. Sta. Bul. 270. 1945.

WHEAT DWARF BUNT DEPRESSED BY COMMON BUNT[1]

R. H. Bamberg, C. S. Holton, H. A. Rodenhiser, and R. W. Woodward[2]

INTRODUCTION

Common bunt (*Tilletia foetida* (Wallr.) Liro and *T. caries* (DC.) Tul.) and dwarf bunt (*T. caries*)[3,4,5] frequently occur in the same wheat field but only occasionally do both kinds develop on the same wheat plant. That the two kinds do not occur together on the same plant more commonly suggests that their developmental processes are antagonistic to each other, either at the time of infection or during development within the host. The results of experiments to determine whether there is a depressing effect of one of these kinds of bunt upon the other are presented in this paper.

MATERIALS AND METHODS

Four tests were made, one of which was in Utah and the others in Montana. Since dwarf bunt cannot be produced by seed inoculation these tests were made in commercial wheat fields in Utah and Montana where the soil was naturally contaminated with dwarf bunt spores. Some of the seed that was planted was inoculated with spores of common bunt while some was not inoculated. Inoculated and noninoculated seed was planted in adjacent rows in the dwarf bunt-infested soil. In the one test in Utah the percentage of smut was determined on the basis of plant counts and in the others in Montana by head counts.

The following wheat varieties were used: Hybrid 128 (C.I. 4512[6]), Oro (C.I. 8220), Golden (C.I. 10063), Yogo (C.I. 8033), and Utah Kanred (C.I. 11608). The inoculum included dwarf bunt (in the soil), and spores of races L-1, 2, 7, and 8 of *Tilletia foetida* and T-2, 10, and 16 of *T. caries*.[7] The powdered inoculum was applied to the seed at the rate of about one gm. spores to 100 gm. seed.

EXPERIMENTAL RESULTS

Utah Kanred wheat and race L–2 were used for the test in Utah.   Plantings were made in dwarf bunt-infested soil near Clarkston, Utah, on October 1, 1941, and duplicated on an adjacent plot 16 days later.   There were 24 eight-foot rows in each planting.   Noninoculated seed was sown in the even-numbered rows and seed inoculated with spores of race L–2 in the odd-numbered rows.   The results of this test are presented in table 1.

High infection was obtained with both dwarf bunt and L–2 on both dates

TABLE 1.—*Effect of Tilletia foetida (race L–2) on the development of dwarf bunt in Utah Kanred wheat, Clarkston, Utah, 1941–42*

Row No.	Inoculum on seed	Percentage of plants in the first planting infected with			Percentage of plants in the second planting infected with		
		Dwarf bunt	Race L–2	Dwarf bunt and race L–2	Dwarf bunt	Race L–2	Dwarf bunt and race L–2
1	L–2	25	53	3	19	61	3
2	None	78	0	0	62	0	0
3	L–2	12	65	12	16	61	3
4	None	71	0	0	71	0	0
5	L–2	23	63	0	11	72	1
6	None	75	0	0	70	0	0
7	L–2	44	20	0	11	66	6
8	None	9	0	0	67	0	0
9	L–2	9	70	7	10	71	3
10	None	42	0	0	70	0	0
11	L–2	12	68	0	6	73	6
12	None	83	0	0	88	0	0
13	L–2	11	71	11	4	75	0
14	None	72	0	0	48	0	0
15	L–2	10	84	4	5	74	1
16	None	76	0	0	56	0	0
17	L–2	12	77	6	3	79	0
18	None	75	0	0	16	0	0
19	L–2	12	75	8	1	80	4
20	None	56	0	0	46	0	0
21	L–2	20	58	9	0	75	2
22	None	26	0	0	16	0	0
23	L–2	18	66	7	0	88	3
24	None	63	0	0	11	0	0
Average	L–2	17	64	6	7	73	3
	None	61	0	0	51	0	0

of seeding.   In the first planting, the rows sown with clean seed averaged 61 per cent dwarf bunt, while those sown with seed inoculated with L–2 averaged 17 per cent dwarf bunt and 64 per cent common bunt.   This reduction of 44 per cent in the dwarf bunt was duplicated in the second planting, thus indicating a definite depressing influence of L–2 on the dwarf bunt.   As shown in table 1, this depressing effect on dwarf bunt was consistent except for one reversal in rows 7 and 8 of the first planting.

In the rows with common bunt (L–2), a small percentage of the plants

had both common and dwarf bunt. In a few cases, both kinds were found in the same plant, the same spike, or even in the same bunt ball, as evidenced by differences in culm height, spore ball characters, and chlamydospore markings. The rarity with which this occurred is further evidence that dwarf bunt does not readily develop in plants infected with race L–2 of common bunt.

The first test in Montana was designed to determine whether some races of common bunt are more effective than others in suppressing dwarf bunt. Hybrid 128 was inoculated with 5 races to which it is susceptible. Oro was inoculated with 2 races to which it is susceptible and with one (T–10) to which it is resistant. Two rows of Hybrid 128 and one row of Oro were grown for checks and all rows were planted in duplicate in each of two fields. The results are summarized in table 2.

TABLE 2.—*Data showing the depressing effect of several races of Tilletia caries (races T–2, 10, and 16) and T. foetida (races L–1 and 8) on the development of dwarf bunt in winter wheat, Bozeman, Montana, 1943–44*

Variety	Inoculum on seed	Percentage of heads with	
		Dwarf bunt	Common bunt
Hybrid 128	L–1	6	88
	L–8	3	92
	None	18	0
	T–10	3	92
	T–16	3	92
	None	23	1
	T–2	6	86
Oro	L–8	0	65
	None	20	0
	T–16	2	65
	T–10	5	5

The incidence of dwarf bunt the year of this test was relatively low. However, a consistently lesser amount occurred in rows with common bunt, regardless of the race used for inoculum or the variety inoculated (Table 2). This was true even on Oro inoculated with race T–10, to which it is resistant. In this test the smut percentages were determined on the basis of total heads per row. Consequently, no observations were made on the simultaneous occurrence of the two kinds of smut in the same plant.

In the second test in Montana, the varieties Golden and Yogo were inoculated with races L–7 and L–8, respectively. Each inoculation was replicated 8 times and each replication had a check row. Plantings were made in the fall of 1944 on each of two farms in dwarf bunt-infested soil. Very little dwarf bunt developed and no data were taken. This test was repeated the following year and the results are presented in table 3.

There was a marked depressing influence of the common bunt on dwarf bunt in both Golden and Yogo. The eight noninoculated rows of Golden had an average of 57 per cent dwarf bunt and no common bunt, in contrast with an average of 14 per cent dwarf bunt and 70 per cent common bunt in

the inoculated rows. Similarly, the noninoculated rows of Yogo had an average of 59 per cent dwarf bunt and no common bunt in contrast with 9 per cent dwarf bunt and 68 per cent common bunt in the inoculated rows. The depressing influence of the common bunt races L-7 and L-8 on dwarf bunt was consistent in all replications with both varieties.

TABLE 3.—*Data showing the depressing effect of races 7 and 8 of Tilletia foetida on the development of dwarf bunt in winter wheat, Bozeman, Montana, 1945–46*

Variety	Inoculum on seed	Total heads	Percentage of heads with	
			Dwarf bunt	Common bunt
		No.		
Golden	L-7	140	18	69
	None	252	47	0
	L-7	124	14	68
	None	237	49	0
	L-7	142	12	71
	None	238	55	0
	L-7	175	15	67
	None	292	54	0
	L-7	161	7	79
	None	307	67	0
	L-7	182	16	69
	None	285	59	0
	L-7	164	14	72
	None	272	65	0
	L-7	171	13	62
	None	284	59	0
Average	L-7	157	14	70
	None	271	57	0
Yogo	L-8	220	9	64
	None	381	55	0
	L-8	365	9	75
	None	435	70	0
	L-8	300	11	67
	None	413	38	0
	L-8	307	8	77
	None	410	69	0
	L-8	304	6	79
	None	377	68	0
	L-8	230	12	60
	None	301	53	0
	L-8	400	8	47
	None	397	56	0
	L-8	413	6	77
	None	328	66	0
Average	L-8	317	9	68
	None	380	59	0

As reported previously, dwarf bunt stimulates tillering in wheat. There is further evidence of this in the record of number of heads in individual rows in table 3. That is, Golden averaged 157 heads in the rows inoculated with common bunt and 271 heads in those with dwarf bunt only. The difference was less striking in Yogo, with averages of 317 and 380 heads, respectively, for the rows inoculated with common bunt and those having

dwarf bunt only. Unexplainable is the fact that two of the noninoculated rows of Yogo had fewer heads than the adjacent inoculated rows. Otherwise there was a marked increase in the number of heads per row, directly attributable to the tiller-stimulating effect of dwarf bunt.

In the data presented there is ample evidence that common bunt has a depressing effect on dwarf bunt development. The nature of this depressant action is not known. By reason of close proximity to the embryo, the common bunt spores had first opportunity to infect. This did not, however, decrease the opportunity for dwarf bunt infection. Apparently, however, it made conditions for dwarf bunt infection less favorable, either by preventing entrance of the infection hyphae or by suppressing parasitic development and sporulation on the host. Under some conditions both kinds of bunt can develop in the same plant (Table 1). This occurred only occasionally, despite its numerous opportunities. Consequently it would seem safe to conclude that physiologic races of *Tilletia caries* and *T. foetida* that cause the common bunt exert a deterring influence on dwarf bunt in the same plant. The races of *T. caries* and *T. foetida* used in these experiments seemed to be about equally effective in inhibiting dwarf bunt.

SUMMARY

Common bunt of wheat was found to have a depressing influence on the development of dwarf bunt. This influence was exercised by all races and on all varieties tested. The nature of this influence is not known.

Evidence is presented to substantiate previous reports that dwarf bunt stimulates tillering in the infected wheat plants.

U. S. DEPARTMENT OF AGRICULTURE
  AND
AGRICULTURAL EXPERIMENT STATIONS OF MONTANA, WASHINGTON,
  AND UTAH

VIRUS INHIBITION BY EXTRACTS OF SPINACH

J. E. KUNTZ AND J. C. WALKER

INTRODUCTION

In the course of studies on viruses of cabbage (*Brassica oleracea* var. *capitata* L.), it was noted that the strain of *Turnip virus 1* designated as cabbage virus A was infectious on spinach (*Spinacia oleracea* L.) (13). While this virus was not recovered by mechanical means from infected spinach, it was transferred readily by aphids. These observations raised the question as to whether there was released into spinach juice upon extraction a substance which inhibited the infectivity of the virus.

Many chemicals have been shown to inhibit the infectivity of viruses in the juice extracted from infected plants. Space does not permit a review of the literature on this subject here. It is worthy of attention, however, that in 1925 Duggar and Armstrong (4) reported that the crude extract of pokeweed (*Phytolacca decandra* L.) markedly inhibited the infectivity of the ordinary tobacco mosaic virus (*Tobacco virus 1* Johnson). Doolittle and Walker (3) reported that they were unable to recover the ordinary cucumber mosaic virus (*Cucumber virus 1* Johnson) from infected pokeweed plants by mechanical means although they did so readily with aphids. Johnson (6) observed that the extract of *Phytolacca rigida* Small almost completely inhibited the tobacco mosaic virus even after aging *in vitro* for "long periods." Others have noted that extracts of pokeweed and of a number of other species are inhibitive to certain viruses (1, 2). Of special interest are the inhibitory effects of extracts of several species of the Chenopodiaceae. Robbins (12) and Jones (8), each failed to recover the virus of sugar-beet mosaic from infected plants by mechanical extraction of the juice. Rawlins and Tompkins (11), however, were able to transmit the virus when they used carborundum as an abrasive. Grant (5) demonstrated that although sugar beet (*Beta vulgaris* L.), Swiss chard (*B. vulgaris* var. *cicla* L.), and spinach (*Spinacia oleracea* L.) were susceptible to ordinary tobacco-mosaic virus, the extracts from healthy plants of the three species greatly reduced the infectivity of the virus in juice extracted from infected tobacco plants. This inhibitive property was reduced greatly by dilution as well as by heating of the plant extracts. Johnson and Grant (7) noted irregularity in amount of infection with spinach juice containing the tobacco ring-spot virus.

This investigation is concerned primarily with the nature of the inhibitive property of spinach extract upon the tobacco-mosaic virus and the cabbage A strain of the turnip-mosaic virus. The inhibitive property of spinach extract was also compared with that of beet and chard. The reactions of the two viruses to spinach extract were compared with those of tobacco

ringspot, latent potato ringspot, necrotic potato ringspot, and cucumber mosaic viruses.

Cultures of all viruses used were maintained in systemically infected host plants kept in insect-proof cages in the greenhouse. The tobacco-mosaic virus, supplied by James Johnson, was maintained on tobacco and concentration of the virus in extracted juice was determined by the local-lesion method on *Nicotiana glutinosa* L. The cabbage-mosaic virus, isolated from naturally infected cabbage, was maintained on Jersey Queen cabbage plants and virus concentration determined by the local-lesion method on tobacco. The cucumber-mosaic virus was taken from naturally infected spinach and was maintained on tobacco and its concentration in juice tested by the local-lesion method on *Amaranthus caudatus* L. The tobacco-ringspot virus, secured from B. M. Duggar, was maintained on tobacco and was tested by the local-lesion method on the same host. The common potato-ringspot virus, which is latent in varieties of potato commonly used in the United States, and a second strain described by Larson (10), which causes necrosis in the variety Chippewa, were secured from R. H. Larson. They were maintained on tobacco and tested by the local-lesion method on the same host. They are designated in this paper as latent potato-ringspot virus and necrotic potato-ringspot virus, respectively.

Healthy plants were grown in a virus-free greenhouse which was kept free of insects by frequent fumigation. Uniform, vigorous plants were selected for virus concentration tests. Leaf tissue of infected plants was macerated in a mortar or by means of a vegetable grinder and the crude infectious juice was extracted from the pulp by squeezing through two layers of sterile gauze or by means by a hydraulic press. The crude juice was centrifuged and the supernatant liquid used. Spinach extract was made from greenhouse-grown plants or from fresh market supply. It was usually extracted in the same manner as infectious juice of virus-infected plants. In some cases a large quantity of spinach extract was made by freezing leaf tissue and filtering the juice through an asbestos-celite filter cake. The extract was stored at 4° C. for future use.

To determine relative concentration of a given virus in a series of juices, host plants were arranged according to a chosen experimental design, labeled, and the surplus leaves and growing point of each plant removed at least one day prior to inoculation. The Latin square design was used whenever possible. Finely powdered carborundum was lightly and evenly dusted over the entire leaf surfaces. In all cases one half of each leaf was inoculated with the test juice and the opposite half with a standard control juice. Inoculation was carried out with sterile glass spatulas of uniform surface area. While the half-leaf was being supported on a folded pad of absorbent paper toweling in one hand, the glass spatula, in the other hand, was dipped once into the inoculum contained in an evaporating dish and

rubbed once, lightly, over the leaf to distribute the inoculum. This preliminary rubbing was followed by two more rubs from the midrib outward with uniform pressure and movement so as to avoid excessive leaf injury and carry any excess inoculum out onto the inoculum pad. A toweling pad was discarded whenever it became damp and always after the final half-leaf inoculation of every treatment. A given test preparation was inoculated to the left half of each leaf of the plants in the first replicate and on alternate sides of the leaves in succeeding replicates. After each test sample of juice was inoculated, the control juice was inoculated to each opposite half-leaf. Inoculated plants were incubated for suitable periods at temperatures most conducive to lesion formation by the respective viruses. When lesion development was sufficiently advanced, detached leaves were placed on a ruled window of an electrically lighted counting box and the number of lesions was recorded with the aid of a mechanical counter.

INHIBITIVE EFFECT OF THE EXTRACTS OF SPINACH, BEET, AND SWISS CHARD

A mixture of equal amounts of spinach extract and the crude juice of cabbage infected with the cabbage-mosaic virus was thoroughly agitated

TABLE 1.—*Average number of lesions per half-leaf of test plants inoculated with untreated infectious juice of cabbage and tobacco and with juice of each treated with equal parts of spinach extract*

Trial No.	Infectious cabbage juice		Infectious tobacco juice	
	Treated	Untreated	Treated	Untreated
1	0	35	0	34
2	0	56	0	11
3	0	44	3	107
4	0	31	0	37
5	0	50	0	25
6	0	42	1	57
7	0	14	......	......

for five minutes and inoculated to half-leaves of actively growing tobacco plants. As a control, the opposite half-leaves of each plant were inoculated with a mixture of equal amounts of distilled water and the infectious cabbage juice. Similarly, juice from tobacco plants infected with the tobacco-mosaic virus was diluted 1–10 with distilled water and a portion of this dilution was mixed with equal parts of spinach extract; the remainder was diluted further with equal parts of distilled water. After thorough agitation the mixtures were inoculated, respectively to half-leaves of *Nicotiana glutinosa*.

The average number of lesions per half-leaf from several trials with infectious juice of cabbage and of tobacco is given in table 1. It is clear that the infectivity of the cabbage-mosaic virus was completely inhibited by this treatment in each trial, while the tobacco-mosaic virus was completely inhibited in four trials and nearly so in two trials.

TABLE 2.—*The inhibitive properties of extracts from leaves of garden beet, sugar beet, and Swiss chard compared with spinach extract*

Source of extract	Ave. no. of lesions per half-leaf inoculated with treated and untreated infectious juice of virus indicated			
	Tobacco mosaic		Cabbage mosaic	
	Treated	Untreated	Treated	Untreated
Spinach	2	69	0	81
Garden beet	1	95	. .	....
Sugar beet	4	63	0	71
Swiss chard	6	82	0	70

The extracts of leaves of garden beet, sugar beet, and Swiss chard were compared with that of spinach in like manner on the tobacco and cabbage mosaic viruses. The results are given in table 2. It is evident that the extracts were equal to that of spinach in their inhibitive properties.

INHIBITIVE EFFECT OF SPINACH EXTRACT ON SEVERAL VIRUSES

In order to determine whether the inhibitive effect of spinach extract was specific to the tobacco and cabbage mosaic viruses, the tests were extended to several other viruses. The virus concentration of treated and untreated juice of tobacco systemically infected with the tobacco-ringspot virus, the latent potato-ringspot virus, and the necrotic-ringspot virus, respectively, was determined in the usual way by the number of local lesions on half-leaves of tobacco. Similar preparations from tobacco infected with the cucumber-mosaic virus were inoculated to half-leaves of *Amaranthus caudatus*. These virus suspensions were compared with those containing tobacco-mosaic virus and cabbage-mosaic virus, respectively. Undiluted juices were mixed with equal parts of spinach extract except for those containing tobacco mosaic and necrotic potato-ringspot viruses, which were diluted with 10 parts of distilled water before mixing with spinach extract. The results are given in table 3. At the concentration used, spinach extract was about equally effective against all six viruses.

TABLE 3.—*Effect of spinach extract upon infectivity of several plant viruses*

Virus	Ave. no. of lesions per half-leaf inoculated with untreated juice and juice mixed in equal parts with spinach extract	
	Treated juice	Untreated juice
Tobacco mosaic	1	45
Cabbage mosaic	0	39
Tobacco ringspot	0	93
Latent potato ringspot	0	58
Necrotic potato ringspot	0	481
Cucumber mosaic	1	21

RATE OF THE INHIBITIVE REACTION

Preliminary experiments indicated that the inhibition of virus infectivity was instantaneous and that no further reduction occurred on standing. In additional experiments, spinach extract diluted 1:5 with distilled water and infectious juice containing tobacco-mosaic virus diluted 1:10 were mixed in equal proportions.   Ten-cc. aliquots of this mixture were stored in plugged test tubes at 20° C. and half-leaves of *Nicotiana glutinosa* were inoculated after definite periods of incubation.   Average numbers of lesions per half-leaf are recorded in table 4. · The results substantiate those of earlier experiments in that the inhibition did not increase on standing. Moreover, little or no reactivation of the virus occurred during the four-hour incubation period.

TABLE 4.—*Comparative inhibitive effects of spinach extract at various intervals after mixture with juice containing tobacco mosaic virus*

Interval between mixing juice and extract and inoculation of test plants	Ave. no. of lesions per half-leaf inoculated with treated and untreated juice	
	Treated	Untreated
Hours		
0.0	5	95
0.5	11	134
1.0	13	154
2.0	16	152
4.0	16	196

LONGEVITY OF THE INHIBITIVE PROPERTY OF SPINACH, BEET, AND CHARD EXTRACTS IN VITRO

Certain experiments were set up to study the longevity of the inhibitive property of spinach extract *in vitro*.   Five-cc. aliquots of the extract stored at 20° C. in cotton-stoppered test tubes were removed at various intervals up to six weeks and mixed in equal proportions with freshly extracted cabbage juice containing the cabbage-mosaic virus.   Half-leaves of tobacco were inoculated with treated juice and with controls consisting of infectious juice diluted with equal parts of distilled water.   In all cases, the aged spinach extract completely inhibited virus infectivity.   When leaves and stems of spinach were stored in manila bags at 4° C. for six months, after which period the leaf tissue was in many cases dry and somewhat decomposed, the extract still completely inhibited infectivity of the cabbage-mosaic virus.   To determine whether any reduction in the inhibitory effect would result from more rapid oxidation, air was continuously bubbled through spinach extract in a 12-inch glass column approximately 6 mm. in diameter for 24 hours.   The extract was still completely inhibitive toward the cabbage-mosaic virus.   In another experiment spinach extract stored for approximately 15 months at room temperature showed no significant reduction in inhibitory property toward the tobacco-mosaic virus.   Beet and chard extracts were equally inhibitive to the tobacco-mosaic virus after

storage for 15 months *in vitro*. It was evident that the inhibitive property was quite stable *in vitro*.

EFFECT OF DILUTION OF SPINACH EXTRACT UPON THE INHIBITIVE PROPERTY

To secure a more accurate evaluation of the degree of inhibition, dilutions of the spinach extract with distilled water were made previous to mix-
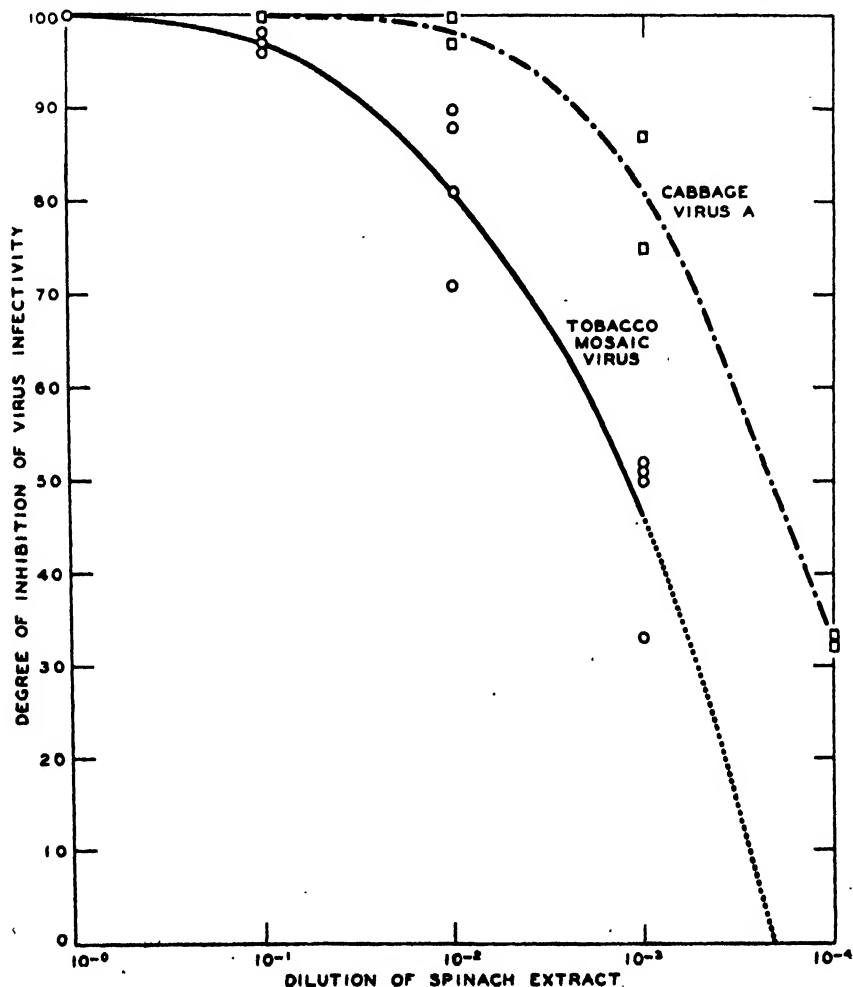
FIG. 1. The effect of dilution of spinach extract on inhibition of the cabbage-mosaic and tobacco-mosaic viruses. Degree of inhibition value was determined by the formula: $100 = \left(\dfrac{\text{number of lesions from treated juice}}{\text{number of lesions from untreated juice}} \times 100\right)$.

ture with infectious cabbage juice and with infectious tobacco juice. In each case the diluted extracts were mixed with equal amounts of infectious juice and the mixtures were inoculated to half-leaves of tobacco or *Nicotiana*

glutinosa. Corresponding half-leaves were inoculated with the respective juice diluted with equal amounts of distilled water.

The effect of dilution on the inhibitory property is shown in figure 1. The reduction in the inhibitory effect by dilution was somewhat more rapid in the case of the tobacco-mosaic virus than with the cabbage-mosaic virus.

Inasmuch as the tobacco-mosaic virus withstood a greater dilution than the inhibitor, an attempt was made to determine the permanency of the inhibitive effect. Infective juice containing the tobacco-mosaic virus was
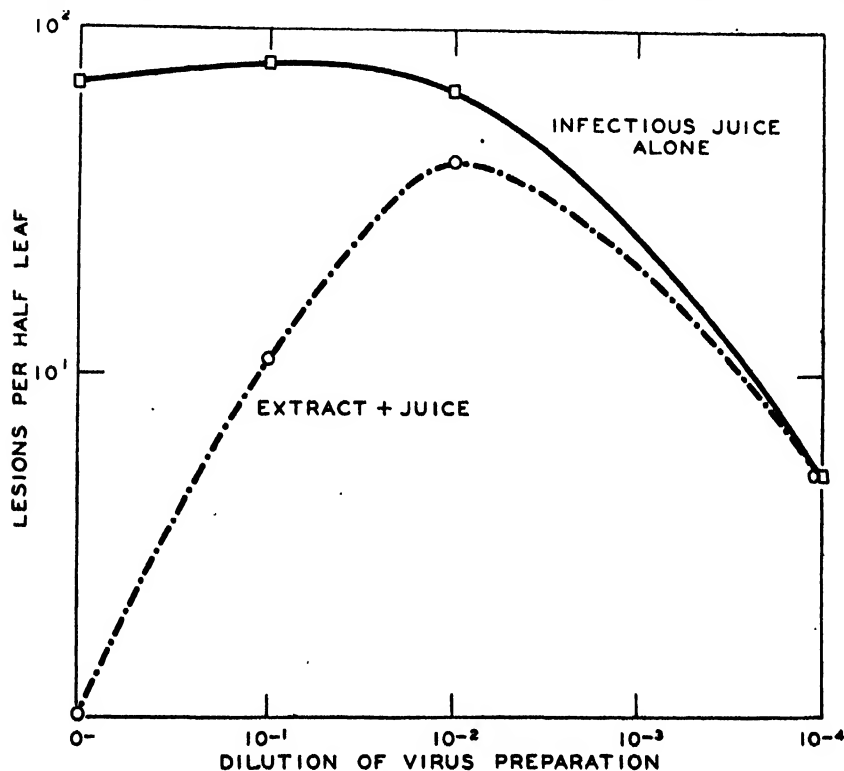
Fig. 2. Restoration of infectivity of the tobacco-mosaic virus by dilution of a mixture of infectious juice and spinach extract.

divided into two portions one of which was mixed with equal parts of spinach extract. This mixture was diluted with distilled water to 10^{-1}, 10^{-2}, and 10^{-4}. The mixture and the various dilutions were each inoculated to six half-leaves of *Nicotiana glutinosa.* As a control, the second portion of the infectious juice was mixed with equal parts of water and then diluted further with water to 10^{-1}, 10^{-2}, and 10^{-4}. These control dilutions were inoculated to the half-leaves opposite those inoculated with the corresponding treated dilutions. The average number of lesions per half-leaf is plotted in figure 2. It is shown that the infectious juice mixed with equal parts of water was highly infectious while the juice mixed with extract was

completely non-infectious. Dilution of the non-infectious mixture resulted in an increase in infectivity of the mixture up to dilution 10^{-2} at which point there appeared to be nearly complete dissociation of the virus and the inhibitive entity. Beyond that point the curve for the mixture followed closely that of the untreated tobacco juice and the reduction in infectivity of the former in dilutions above 10^{-2} was due primarily to the effect of dilution on the concentration of the virus.

ADSORPTION OF THE INHIBITIVE FACTOR

Aliquots of spinach extract were mechanically stirred for five minutes with 10 per cent (by weight) Nuchar W, an activated charcoal. Following agitation, the charcoal-spinach extract mixture was filtered through an asbestos cake on Whatman No. 40 filter paper in a Büchner funnel. The filtrate was mixed in equal proportions with infectious cabbage juice. Another portion of the spinach extract was similarly treated with celite, a

TABLE 5.—*The effect of Nuchar W and celite as adsorptive agents on spinach extract*

Virus	Ave. no. of lesions per half-leaf inoculated with infectious juice treated as indicated (T) and ave. no. of lesions on corresponding half-leaf inoculated with untreated juice (C)					
	Crude spinach extract		Filtrate from spinach extract mixed with celite		Filtrate from spinach extract mixed with Nuchar W	
	T	C	T	C	T	C
Cabbage mosaic	0	46	0	60	0	93
Tobacco mosaic	0	341	10	280	251	253

siliceous adsorbent, and the filtrate likewise mixed in equal proportions with infectious cabbage juice. Controls consisted of equal parts of infectious cabbage juice and distilled water. Half-leaves of tobacco were inoculated. In a similar series of experiments, infectious tobacco juice was treated with the filtrates of the Nuchar W and celite-treated spinach extracts and inoculations were made to half-leaves of *Nicotiana glutinosa*. The results are given in table 5. Neither celite nor Nuchar W affected the inhibitive action of spinach extract on the cabbage-mosaic virus. Celite did not affect the inhibitive action of the spinach extract upon the tobacco-mosaic virus; Nuchar W, however, completely removed the factor inhibitive to the latter virus. This differential reaction indicated that perhaps two inhibitive factors were concerned in the spinach extract, one of which was inhibitive to tobacco mosaic and was adsorbed by Nuchar W. The inhibitory agent was not recovered from the charcoal filter cake by extraction with acid and alkaline solutions of various strengths.

The effect of certain other adsorptive agents on the inhibitory actions of spinach extract on virus activity was tested. Portions of spinach extract were agitated for five minutes with 10 per cent (by weight) of the respective

materials, and the mixtures were filtered through hard filter paper. The effect of the filtrates on the inhibition of virus infectivity was tested by mixing with equal amounts of tobacco-mosaic-virus extract. The ratio of the average number of local lesions per half-leaf produced by the filtrate-treated virus suspensions to that of the controls similarly diluted with water was as follows: Hy-flo Standard Super-cel, 1:100; wood charcoal, 1:100; celite, 4:100; animal charcoal, 6:100; Fuller's earth, 60:100; bone charcoal, 72:100; norite, 125:100; Nuchar W, 140:100. These results showed that Super-cel, wood charcoal, celite, and animal charcoal had very little effect on the inhibitory agent; Fuller's earth and bone charcoal apparently removed a part of the inhibitory agent; and norite and Nuchar W not only removed the inhibitory agent, but also increased the infectivity of the virus preparation.

Since the component of spinach extract which is inhibitive to the tobacco-mosaic virus could be removed by certain adsorptive agents, an experiment

TABLE 6.—*Restoration of tobacco-mosaic-virus infectivity by differential adsorption*

Treatment	Average number of lesions per half-leaf inoculated with treatment preparation indicated (T) and untreated (C)	
	T	C
Infectious juice diluted 1:1 with water and then treated with norite	172	159
Infectious juice mixed with equal parts of spinach extract and then treated with norite	176	182
Spinach extract treated with norite and then mixed with equal parts of juice	159	162

was designed to restore the infectivity to a noninfectious mixture by differential adsorption. Infectious tobacco juice was mixed in equal parts with spinach extract. Twenty cc. of this noninfectious mixture was thoroughly beaten for five minutes with two gm. of norite and the norite was then removed by filtration through hard filter paper. An aliquot of spinach extract alone was treated in a similar manner with norite and filtered previous to mixing with infectious juice. To allow for any effect of the norite on the tobacco-mosaic virus itself, infectious juice was diluted with equal parts of distilled water and the preparation likewise treated with norite and filtered. The filtrate or the filtrate mixed with infectious juice was inoculated to half-leaves of *Nicotiana glutinosa;* controls consisted of untreated infectious juice diluted 1:1 with distilled water. The results (Table 6) show that norite was effective not only in removing the inhibitor from spinach extract, but also in restoring the infectivity of a practically noninfectious mixture. When the adsorptive agent was applied to infective tobacco juice it increased the number of lesions over that of the control, suggesting that there might be a slight amount of an inhibitor in the tobacco juice. In a second experiment it was shown that practically the same

results were secured when the norite was filtered off before inoculation as when it was left in the mixture at the time of inoculation.

Norite treatment of a noninfectious mixture of spinach extract and cabbage-mosaic juice did not restore infectivity. This was in accord with a previous observation that adsorptive agents did not remove the substance inhibitive to the cabbage-mosaic virus. On the other hand norite readily removed the inhibitive effect of spinch extract on the necrotic potato-ring-spot virus. When a mixture of tobacco juice containing this virus and spinach extract was treated with this adsorptive agent more than 1000 lesions per half-leaf were produced by the filtrate, while none was produced by the untreated control mixture.

EFFECT OF HEATING SPINACH EXTRACT UPON THE INHIBITIVE PROPERTY

In a study of the effect of heating spinach extract upon its inhibitive property the former was first filtered through an asbestos-celite cake to

TABLE 7.—*Effect of heat upon the inhibitive property of spinach extract*

Treatment temperature (12 min.) °C.	Average no. of local lesions per half-leaf inoculated with preparations of heated spinach extract and infectious juice (T) and infectious juice alone (C), containing			
	Tobacco-mosaic virus		Cabbage-mosaic virus	
	T	C	T	C
55	0	80	0	21
60	0	112	0	14
65	80	101	0	38
70	103	101	0	20
80	111	114	0	15
90	85	75	0	12
100	101	95	0	24
125ª	....	....	0	75

ª Treated for 15 minutes.

clarify the extract. Two-cc. aliquots of clarified spinach extract were drawn into thin-walled tubes, one end of each of which was sealed off. Three filled tubes were then inserted into a continually agitated water bath held within 0.1° C. of the desired temperature. After immersion for 12 minutes the tubes were cooled in running water, the drawn tips were broken off, and the contents of all these tubes forced into a 10-cc. centrifuge tube. The coagulum from the heat precipitation was thrown down in a centrifuge and the supernatant liquid was pipetted into another tube. The original reaction of the spinach extract, about pH 6.8, was not changed appreciably by the heat treatment. By this method a range of treatments at various temperatures was carried out.

The heated extracts were mixed in equal parts with tobacco juice containing the tobacco-mosaic virus and with cabbage juice containing the cabbage-mosaic virus, and half-leaves of the respective test plants were inoculated. Controls of the respective virus juices diluted with equal parts

of distilled water were inoculated to opposite half-leaves.   Data from a representative series are given in table 7.

The two viruses did not respond to heated spinach extract in the same manner.   The inhibitive property of the extract toward tobacco-mosaic
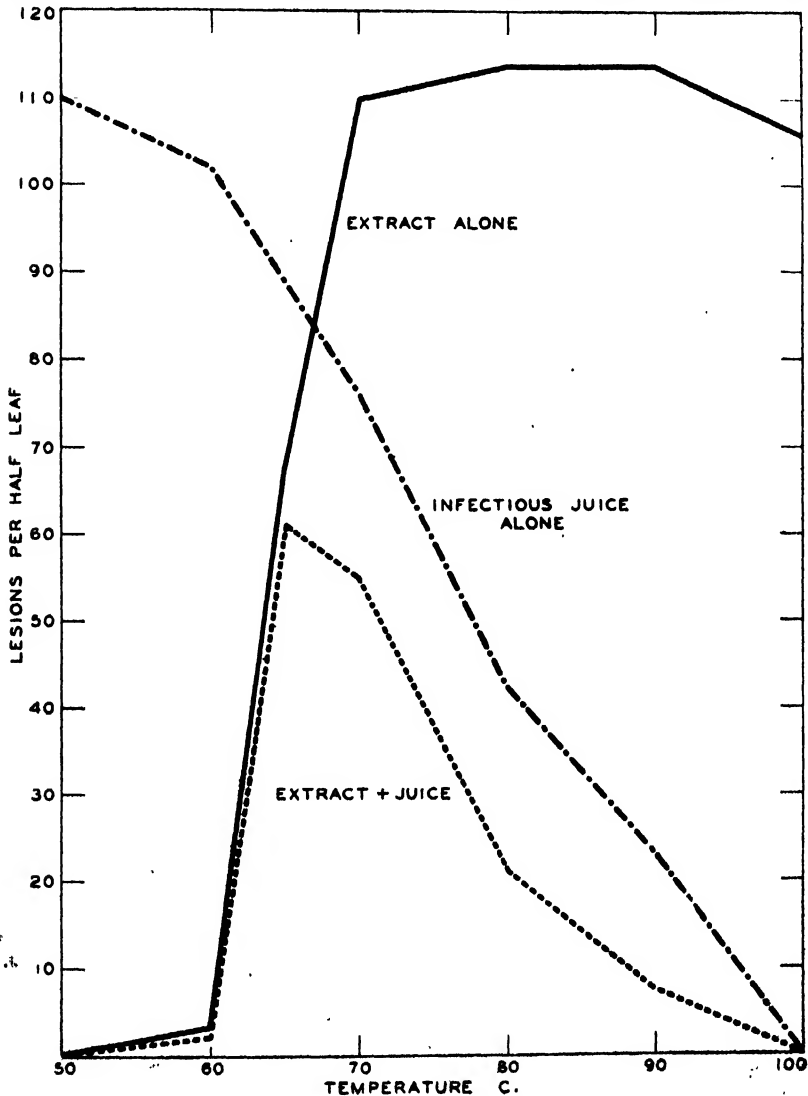
FIG. 3.   The effect of various temperatures for 10-minute intervals on the infectivity of (1) a mixture of spinach extract and infectious tobacco-mosaic juice, (2) spinach extract alone, later mixed with infectious juice, (3) the infectious juice mixed with distilled water.

virus was thermolabile, being greatly reduced by exposure to 65° C. for 10 minutes and completely destroyed at 70°.   On the other hand, heat did not destroy the inhibitive property of the extract toward the cabbage-mosaic

virus when exposed at 125° for 15 minutes. In most experiments in which the heat treatments were repeated similar results were obtained. In some cases the inhibitive effect of spinach extract toward the cabbage-mosaic virus was reduced by approximately one half when the former was heated at 100° for 15 minutes. The results indicated that either there were two distinct inhibitive substances in spinach extract, one thermostabile and one thermolabile, or that there was one substance which was destroyed in part by heat and in the resultant dilution of that substance the cabbage virus, being more sensitive than the tobacco virus, was completely inhibited.

In order to determine whether the infectivity of a noninfectious mixture of spinach extract and infectious tobacco juice could be restored by heating, spinach extract was mixed in equal proportions with infectious tobacco juice and 5-cc. aliquots of the mixture heated in test tubes immersed in a constant-temperature water bath for 10-minute intervals at various temperatures. Following treatment, the preparation was centrifuged and half-leaves of *Nicotiana glutinosa* were inoculated with the supernatant liquid. Two controls were employed. One control consisted of aliquots of spinach extract similarly heated and then mixed with the infectious juice. A second control consisted of the infectious juice mixed with equal parts of distilled water and similarly heated. The results of a representative experiment are presented graphically in figure 3. The spinach extract alone lost its inhibitive property completely at 70° C. With the infectious juice alone, number of lesions per half-leaf as a function of temperature followed a descending straight line curve. When the mixture of spinach extract and infectious juice was heated there was evidence of some dissociation of inhibitor and virus at 60°. At 65° where there was a discrepancy of about 30 lesions per half-leaf between the mixture and the infectious juice heated alone, the dissociation appeared to be nearly complete. Increase in temperature above 65° had the same effect on the virus in the mixture as it did on infectious juice alone. It was clear from these experiments that the infectivity of a mixture of infectious tobacco juice and spinach extract could be restored almost completely by heating to 65° C. Since infectious cabbage juice is inactivated at 56° to 58° for 10 minutes (13), it is obvious that this virus would be destroyed at a lower temperature than the inhibitor.

EFFECT OF DIALYSIS UPON THE INHIBITIVE PROPERTY

Dialysis of celite-clarified spinach juice was carried out against frequently changed distilled water and flowing tap water by means of a modified Kunitz-Simms apparatus (9). Twenty-cc. aliquots of spinach juice were dialysed through a Visking sausage casing against four 300-cc. portions of distilled water and likewise against running tap water over a four-day period with the water cylinders mounted on a mechanical rocker. Both preparations contained in the sausage-casing bags were tested for inhibitory properties, as was the dialysed material after 1200 cc. of distilled water had been reconcentrated to the original volume by vacuum distillation at room

temperature.   The three respective preparations were mixed in equal pro-
portions with infectious tobacco juice .containing the tobacco-mosaic virus
and were inoculated to half-leaves of *Nicotiana glutinosa*.   In another ex-
periment, a similar dialysis procedure was carried out with a cellulose
membrane.   In this case, 25 cc. of spinach extract was dialysed against
flowing tap water and also against a 150-cc. portion of distilled water over
a 2-day period with only occasional agitation.   The 150-cc. portion of dis-
tilled water was used without reconcentration.   Inocula were prepared in
the ratio of 1:1, using infectious cabbage juice, and inoculated to half-
leaves of tobacco.   The results of both experiments are given in table 8.

On the basis of the results secured with the dialysable portion of spinach
extract on the infectivity of the cabbage virus, it was first thought that the

TABLE 8.—*The effect of dialysis upon the inhibitive factor in spinach extract*

Membrane used in dialysis	Time in days	Virus	Average no. of lesions per half-leaf inoculated with juice treated with fraction indicated (T) and with untreated (C) juice					
			Dialysis into distilled water				Dialysis into tap water	
			Dialysed fraction		Non-dialysed fraction		Non-dialysed fraction	
			T	C	T	C	T	C
Sausage casing, Visking	4	Tobacco mosaic	234	217	3	314	6	365
Cellulose casing, Sargent	2	Cabbage mosaic	9	80	0	90	0	90

inhibitive agent was slowly dialysable through the cellulose casing and that
at the time the tests were made, an equilibrium had been established.   Ap-
parently enough inhibitive component had diffused outwardly to cause a 90
per cent reduction in infectivity despite the dilution effect of the distilled
water, while yet enough inhibitive factor remained within the sac to com-
pletely inhibit infectivity.

The results secured with the standard sausage-casing membrane using
tobacco-mosaic virus as an indicator of inhibition indicated that no dialysis
of the inhibitive agent had taken place,—even when the outer distilled water
containing the dialysed portion had been reconcentrated to the original
volume. On the other hand, if the virus preparation had been diluted
further before treatment some inhibitive effect might have been noticed;
still, had any appreciable dialysis taken place, the lesion count would have
far exceeded that of a mixture of infectious tobacco juice and untreated
spinach extract which gave an average of four lesions per half-leaf.   The
factor inhibitive to tobacco-mosaic virus appeared, therefore, to be non-
dialysable or very slowly so.

In accord with the differential thermostability of spinach extract re-
flected in the inhibition of infectivity of the tobacco-mosaic virus and the

cabbage-mosaic virus, a similar differentiating reaction was found in the fact
that the factor inhibitive to the tobacco-mosaic virus did not dialyse while
that inhibitive to the cabbage-mosaic virus alone did.   This further indicated
that two inhibitors might be present in spinach extract one of which was
dialysable, while the other was nondialysable.   On the other hand the results
might be explained on basis of partial dialysis of the inhibitor and greater
sensitivity of the cabbage-mosaic virus to it.
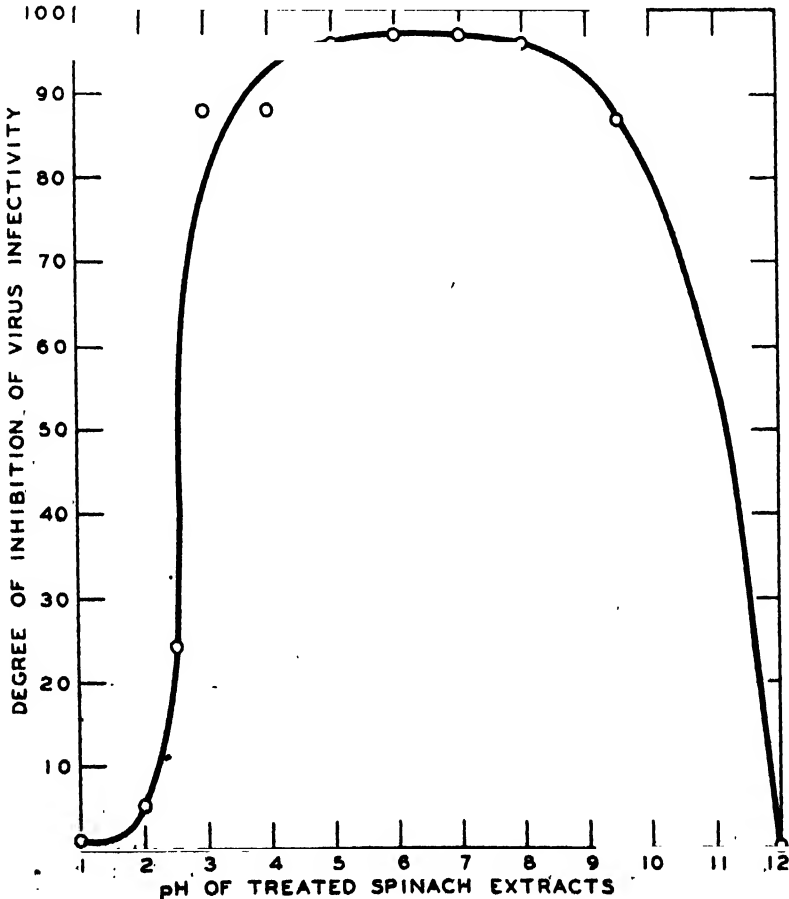
Fig. 4.   The effect of hydrogen-ion concentration on the inhibition of virus infec-
tivity by spinach extracts after readjustment to pH 7.

EFFECT OF HYDROGEN-ION CONCENTRATION ON THE INHIBITIVE PROPERTY

An experiment was designed to determine the effect of hydrogen-ion
concentration on the inhibitory action of spinach extract.   Adjustments in
reaction of spinach extract ranging from pH 1 to 12 were made by adding
the necessary amounts of N/1 HCl or N/1 NaOH to 4-cc. aliquots of spinach
extract during constant agitation.   Sufficient distilled water was added to
the treated spinach extracts to equalize the different dilutions caused by the

addition of varying amounts of acid or base, as the case might be. Hydrogen-ion determinations were made by means of a Beckman pH meter during titration and immediately following a 24-hour treatment period at the desired pH. No preparation varied more than 0.2 of a pH unit during the treatment. All treated extracts were readjusted to pH 7, dilution effects again being equalized, and the inocula were prepared by mixing equal parts of the neutral extract with infectious tobacco juice containing the tobacco-mosaic virus. Inoculations were made immediately on half-leaves of *Nicotiana glutinosa*. A standard control consisting of equal parts of distilled water and crude infectious juice was inoculated on each opposite half-leaf. The degree of inhibition of virus infectivity by the treated extracts is shown in figure 4. Results indicated that the inhibitive factor was destroyed in extremely alkaline and extremely acid solutions. When the pH was changed to points within the range of pH 3 to 9.5 and readjusted to neutral there was no appreciable change in the inhibitory effect. No tests were made of the influence of the pH of spinach extract on the cabbage-mosaic virus.

<div style="text-align:center">PRECIPITATION OF THE INHIBITORS</div>

Spinach extract, infectious tobacco juice, and a mixture of the two were each precipitated by bringing them to 50 per cent ethyl alcohol through the addition of sufficient 95 per cent alcohol. The treated preparations were shaken thoroughly and allowed to stand 2½ hours. The precipitate in each case was thrown down in a centrifuge, the supernatant liquid pipetted off, and the residue washed twice with 50 per cent alcohol. The wash in each case was added to the original supernatant liquid which was twice reconcentrated to a syrupy consistency and finally restored to the original volume with water. The washed precipitate was resuspended in distilled water equal to the original volume. Each component of the treated spinach extract was then mixed with equal parts of tobacco-mosaic juice and the mixture inoculated to half-leaves of *Nicotiana glutinosa*, the control half-leaf in each case being inoculated with infectious tobacco-mosaic juice mixed with equal parts of distilled water. Inoculations of the other preparations were carried out in a similar manner. The results are summarized in table 9. The mixture of extract and juice was completely noninfectious. The precipitate from infectious juice when resuspended in water was nearly as infectious as untreated juice, while the supernatant liquid was also equal to the untreated juice in infectivity. This showed that part of the virus was precipitated and a considerable amount remained in the supernatant liquid. Apparently the original juice had the potentiality of producing many more lesions than could be measured on a single half-leaf. When the spinach extract alone was precipitated the inhibitor was completely removed from the supernatant liquid while it was not released from the precipitate when the latter was resuspended in the original volume of water. When the mixture of juice and extract was precipitated the infectivity of

the supernatant was partially restored and a portion of the virus particles carried down by the precipitate was released upon resuspension of the latter in water. It was shown by this experiment that the factor inhibitive to tobacco-mosaic virus was precipitated completely by 50 per cent alcohol. Moreover the infectivity of the noninfectious mixture of juice and extract was largely restored in the supernatant by precipitation with the same concentration of alcohol.

The supernatant liquid from precipitation of spinach extract with 50 per cent alcohol was tested against infectious cabbage juice. When juice and supernatant liquid were mixed in equal proportions the mixture was completely noninfectious while the cabbage juice control caused an average of 90 lesions per half-leaf. Thus it became clear that the tobacco-mosaic

TABLE 9.—*The effect of alcohol precipitation on tobacco-mosaic juice, and on the inhibitive property of spinach extract*

Preparation of material	Ave. no. of lesions per half-leaf inoculated with treated (T) and untreated (C) juice	
	T	C
Spinach extract mixed with infectious juice	0	147
Precipitate of infectious juice resuspended in water	131	189
Supernatant liquid from precipitated infectious juice reconcentrated to original volume	114	108
Precipitate of spinach extract resuspended in water and mixed with equal parts of infectious juice	154	148
Supernatant liquid from precipitated spinach extract reconcentrated to original volume and mixed with equal parts of infectious juice	148	153
Precipitate of mixture of extract and juice, resuspended in water	55	175
Supernatant liquid from precipitated mixture of extract and juice, reconcentrated to original volume	128	144

inhibitor could be separated from the cabbage-mosaic inhibitor by precipitation with 50 per cent alcohol.

The evidence already presented indicated that two inhibitive entities occurred in spinach extract, each of which, of course, might comprise one or more compounds. One of these entities was inhibitive to the tobacco-mosaic virus. Whether or not it was inhibitive to cabbage-mosaic virus was not ascertained. It was destroyed at 65° C. for 12 minutes; it was non-dialysable; it was precipitated by 50 per cent alcohol. These characteristics suggested that the substance (or substances) in this component were non-crystalloidal, relatively high in molecular weight, and similar to proteins in some respects. The second component, on the other hand, was not inhibitory to tobacco-mosaic virus but decidedly so to the cabbage-mosaic virus. It was not destroyed at 125° C. for 15 minutes; it was dialysable; it was not precipitated by 50 per cent alcohol. These characteristics indicated that the substance (or substances) in this component was crystalloidal.

In view of the fact that free oxalic acid occurs to some extent in spinach

leaves as well as in those of beet and chard the effect of complete precipitation of such acid on the inhibitor was next tested.   Spinach extract was first treated with norite and filtered to remove the tobacco-mosaic inhibitor.   Another sample of extract was heated at 70° C. for 12 minutes and filtered to remove the tobacco-mosaic inhibitor.   To 2-cc. aliquots of the respective filtrates three drops of 10 per cent calcium chloride solution were added and the heavy milky precipitate removed by filtration through an asbestos filter cake.   The original and precipitated filtrates were each mixed in equal parts with cabbage-mosaic juice and inoculated to half-leaves.   The opposite half-leaves were inoculated with a control consisting of the infectious cabbage juice mixed with equal parts of distilled water and treated with the same proportions of calcium-chloride solutions.   The results are given in table 10. It is evident that the cabbage-mosaic inhibitor was completely or almost completely precipitated by calcium chloride.

TABLE 10.—*The effect of calcium chloride on the cabbage-mosaic inhibitor in spinach extract*

Treatment of spinach extract before mixing with infectious cabbage juice	Total no. of lesions on 10 half-leaves inoculated with juice treated with the fraction indicated (T) and juice treated only with calcium chloride (C)	
		C
Treated with norite and filtered	17	94
Treated with norite, filtered, treated with calcium chloride and filtered	70	68
Heated at 70° C. 12 minutes and filtered	0	99
Heated at 70° C. 12 minutes, filtered, treated with calcium · chloride and filtered	74	86

DISCUSSION

The foregoing experiments clearly demonstrate a striking inhibitory effect of crude spinach extract *in vitro* upon the infectivity of tobacco-mosaic and cabbage-mosaic viruses notwithstanding the fact that these viruses may infect spinach and increase in quantity in infected plants.   Little is to be gained in speculation here concerning the physiological and biochemical processes involved in this difference between virus activity in living tissue and virus activity in the presence of extracted plant juice.   In spite of the difficulty encountered in the mechanical transmission of the cabbage-mosaic virus from macerated host tissue, insect vectors readily extract the virus in an active form and transmit it to healthy plants.   Nothing observed throughout this investigation indicates that the inhibitory action of certain plant extracts is due to modifications of the host plant inoculated with a given preparation.   Rather it is indicated that distinct chemical substances present in the extracts of spinach and some other chenopodiaceous plants render the viruses noninfective.   The inhibitive factor apparently remains stable and functional for a long time in non-sterile juice and it is not destroyed by the

usual fermentation processes. Its effect on the virus is immediate, but the infectivity of a noninfectious preparation can be restored by various treatments that separate the virus from the inhibitor.

It is suggested that two inhibitory substances are present in the spinach extract or that two fractions of the same substance occur. The first is thermolabile at 70° C., nondialysable, adsorbed by activated charcoal, unstable in extreme acid and alkaline solutions, precipitated by alcohol, and rendered inactive at dilutions of 1 : 10,000. The infectivity of a noninfectious preparation in which this inhibitor is operative can be restored by submitting the mixture to any of the above treaments. Such properties suggest a material colloidal in nature, of high molecular weight, and proteinaceous in character. The second inhibitory component of spinach extract as measured by its effect on the cabbage-mosaic virus is thermostable, dialysable, not precipitated by alcohol, and only slightly adsorbed by activated charcoals. Moreover its action is irreversible. It is probably crystalloidal and much simpler in chemical structure.

It is evident from this study that the common difficulty in transmitting viruses from infected chenopodiaceous hosts is due to an inhibition of virus infectivity by components of the plant extract. Inhibitors of varying potentialities have been reported to exist in other plants but usually in too low a concentration to be of any significance. On the other hand, these results demonstrate that the concentration of virus in crude infectious plant juice may not necessarily be accurately measured by the local-lesion method. Failure to recover viruses from inoculated plants by mechanical methods is certainly not to be taken as final negative evidence. Undoubtedly the so-called increase in virus concentration of a plant virus by treatment of the infectious extract is often due to the release of the virus from a virus-inhibitor complex.

SUMMARY

The extracts of leaves of spinach, garden beet, sugar beet, and chard, when mixed in equal parts with the infectious juice of tobacco containing the tobacco-mosaic virus (*Tobacco virus 1*) and with the infectious juice of cabbage containing the cabbage-mosaic virus (*Turnip virus 1*), completely or almost completely inhibited the infectivity of those juices. When spinach extract was mixed in equal parts with plant juices containing the tobacco-ringspot virus, the latent potato-ringspot virus, the necrotic potato-ringspot virus, and the cucumber-mosaic virus, the infectivity of those juices was also completely or nearly completely inhibited. The action of the spinach extract was instantaneous and did not increase with time; the extract retained its inhibitive effect at least 15 months at room temperature.

The inhibitive effect upon the tobacco-mosaic and upon the cabbage-mosaic virus was reduced by dilution of the spinach extract. After treatment with norite and with Nuchar W the extract did not measurably inhibit the tobacco-mosaic virus but was still inhibitive to the cabbage-mosaic virus.

Heating of the extract to 70° C. for 10 minutes rendered it noninhibitive to the tobacco virus but when heated to 125° for 15 minutes it still completely inhibited the cabbage virus. The component inhibitive to the tobacco virus did not dialyse in sufficient amount to be measured but the dialysate was inhibitive to the cabbage virus.

When spinach extract was adjusted to various reactions from pH 1 to 12 for 24 hours and the reaction readjusted to pH 7 there was no appreciable change in the inhibitive effect on the tobacco-mosaic virus from pH 3 to pH 9.5; the inhibitive property was destroyed in extremely acid and extremely alkaline solutions.

Spinach extract was diluted with 95 per cent ethyl alcohol to the point where the mixture contained 50 per cent alcohol. After being shaken, allowed to stand 2½ hours, centrifuged, and washed with 50 per cent alcohol the supernatant liquid had no inhibitive effect on tobacco-mosaic virus; the precipitate, moreover, did not release any of the inhibitive material when resuspended in distilled water. When the supernatant liquid was mixed with cabbage-mosaic virus it was completely inhibitive.

When the tobacco-mosaic inhibitive factor was removed from spinach extract by treatment with norite or by heating the extract, and the norite or precipitate removed by filtration, the filtrates were still inhibitive to the cabbage-mosaic virus. When calcium-chloride solution was added to such filtrates and the precipitate was filtered off, the second filtrate was not inhibitive, indicating that the factor inhibitive to the cabbage-mosaic virus had been removed by precipitation with calcium chloride.

UNIVERSITY OF WISCONSIN,
    MADISON, WISCONSIN.

LITERATURE CITED

1. BAWDEN, F. C. Plant viruses and virus diseases. 2nd edit. 294 pp. Waltham, Mass. 1943.
2. ————, and A. KLECZKOWSKI. Protein precipitation and virus inactivation by extracts of strawberry plants. Jour. Pomology 21: 2–7. 1945.
3. DOOLITTLE, S. P., and M. N. WALKER. Further studies on the overwintering and dissemination of cucurbit mosaic. Jour. Agr. Res. [U.S.] 31: 1–58. 1925.
4. DUGGAR, B. M., and J. K. ARMSTRONG. The effect of treating the virus of tobacco mosaic with the juices of various plants. Ann. Mo. Bot. Gard. 12: 359–366. 1925.
5. GRANT, T. J. The host range and behavior of the ordinary tobacco-mosaic virus. Phytopath. 24: 311–336. 1934.
6. JOHNSON, J. Chemical inactivation and the reactivation of a plant virus. Phytopath. 31: 679–701. 1941.
7. ————, and T. J. GRANT. The properties of plant viruses from different host species. Phytopath. 22: 741–757. 1932.
8. JONES, L. K. The mosaic disease of beets. Wash. Agr. Expt. Sta. Bull. 250. 1931.
9. KUNTZ, M., and H. S. SIMMS. Dialysis with stirring. Jour. Gen. Physiol. 11: 641–644. 1928.
10. LARSON, R. H. A foliar mottle and necrosis in Chippewa potatoes associated with infection by a strain of the potato X virus. Phytopath. 33: 1216–1217. 1943.
11. RAWLINS, T. E., and C. M. TOMPKINS. Studies on the effect of carborundum as an abrasive in plant virus inoculations. Phytopath. 26: 578–587. 1936.
12. ROBBINS, W. W. Mosaic disease of sugar beets. Phytopath. 11: 349–365. 1921.
13. WALKER, J. C., F. J. LeBEAU, and G. S. POUND. Viruses associated with cabbage mosaic. Jour. Agr. Res. [U.S.] 70: 379–404. 1945.

CLUBROOT OF TOBACCO: A WOUND–TUMORLIKE GRAFT–TRANSMITTED DISEASE[1]

W. D. V A L L E A U

In August, 1922, specimens of tobacco with enlarged roots were received from Campbell County, Kentucky.   As these plants had some similarity to those attacked by root-knot nematode, they were carefully examined for nematodes, but none was found.   Specimens were sent to Dr. N. A. Cobb on two occasions and both times he reported the disease was not caused by nematodes.   It was given the name "clubroot" because of the similarity of the enlarged roots to clubroot of cabbage.[2]

The disease was observed later near Owensboro, where the root symptoms were associated with peculiar plant characteristics in half-grown plants. The lower leaves were normal in size and appearance but the upper leaves were abnormally short, giving the plant a pyramidal shape.   The growth of the plant was stunted considerably.

In 1946, while plants were being examined for brown root rot, clubroot was found in Fayette, Owen, Boone, and Mason Counties.   The diseased plants were usually growing poorly but could not easily be distinguished from other slow-growing but apparently unaffected plants except by examination of the roots.

Several plants were transplanted from the field to pots in the greenhouse for further study.   One plant was given to Dr. L. M. Black for comparison with his wound-tumor disease which clubroot seemed to resemble closely.

Assuming that the disease was caused by a virus, some tests were made to determine whether the causal agent was systemic.   Evidence that the causal agent was present in the above-ground parts of affected plants was obtained from a cutting of an affected plant and from grafting scions from affected plants on healthy tobacco plants.   On October 10, 1946, a cutting was made from the top of a 2-foot-tall clubroot Burley tobacco plant.   It was planted in a steam-sterilized mixture of sand and soil in a 4-inch pot and placed under a bell jar.   On November 28 the cutting was removed and found to be fairly well rooted.   The roots showed no signs of tumors, but a large tumor ¾ inch in diameter and several small tumors had formed over the cut surface of the cutting.   All of the roots had developed from the large tumor.   The cutting had grown very little, but the growing point curved toward the side on which the large tumor had developed.   Following examination the cutting was planted in a ground bench in the greenhouse for further observation.   On April 3, 1947, approximately 4 months after trans-

planting and a few days after the plant had died, it was dug up for exami-
nation.   There had been little if any growth of the top.   There had been
some development of the roots but it was largely confined to the production
of new roots from the tumors and to the development of a few laterals from
the larger roots.   While the roots at the time of transplanting showed no
tumors, they now had tumors at the points where lateral rootlets were pro-
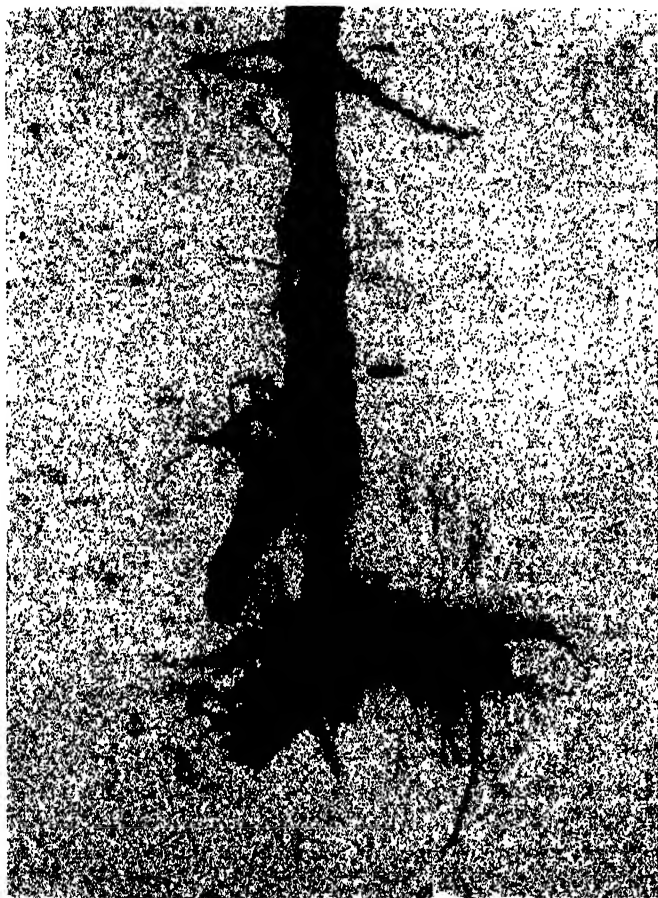
FIG. 1.   Clubroot of Burley tobacco.   A scion from a clubroot Burley tobacco plant
was grafted on a healthy root of Ky 16 Burley.   After union had taken place the plant
was set in a ground bench with the graft union (arrow) 4 inches below the surface.
Tumors may be seen on the scion roots.   The stock root system is a mass of tumors.

duced.   There was no indication that the large tumors on the base of the
cuttings or the tumors on the roots had been caused by root knot nematodes.
   Grafts were made on three Ky 16 Burley plants growing in 4-inch pots,
using scions from three clubroot Burley plants.   All made good unions, but
when the roots were washed about a month after grafting they were found
to have black root rot (*Thielaviopsis basicola*) and to have made but little
root growth.   There were no signs of tumors on the roots.   One of the

plants, following washing and removal of many of the rootlets, was transplanted to sand and left for 5 weeks, when the roots were again washed. The new rootlets appeared normal but the roots from which they grew were irregularly swollen and similar in appearance to a mild natural case of clubroot. A second plant grafted October 18, 1946, was examined November 28, but no tumors were apparent. The plant was then set in a ground bench with the graft union 4 inches below the surface, in the hope that roots might develop from the scion. This plant sent out several shoots that bloomed at a height of about 2 feet and ripened seed. The leaves were small but otherwise normal. On April 4, 1947, 154 days after transplanting, the plant was dug and the roots washed. A few roots had developed from the scion, and these had small tumors at intervals where lateral rootlets developed. The Ky 16 root system, originally healthy, was made up of a few much enlarged and distorted roots definitely of the type found on naturally infected field plants (Fig. 1). Some of the younger roots had small tumors at points where rootlets originated, while the youngest roots appeared normal. It seemed that the tumors on the old roots gradually increased in size until the whole root appeared to be made up of an irregular mass of tumor tissue. A careful examination of the beadlike enlargements on the smaller roots indicated that root knot nematodes were not involved.

In the instance of the cutting and the two grafts the causal agent evidently was present in the growing points of the clubroot plants, because roots that developed from the cutting and from the scion had well-formed tumors. Furthermore, in the two grafted plants the causal agent appeared to have been transmitted from the scion to the previously healthy roots upon which they were grafted.

Because of the similarity of the tumors to those caused by Black's[3] wound-tumor virus and because both diseases appear to be caused by viruses, it is probable that the virus of clubroot is closely related to, if not the same species as, the wound-tumor virus. The virus of the wound-tumor disease is transmitted by the agallian leaf hoppers *Agallia constricta* Van Duzee, *Agallia quadripunctata* (Provancher), and *Agalliopsis novella* (Say). While Black did not test tobacco as one of the possible hosts of the virus, the wound-tumor virus has a very wide host range in which tobacco might be included.[4]

Since this paper was written the writer has seen an abstract of a paper by Trotter[5] in which he describes a disease of Kentucky and Burley tobacco growing in Italy which is very similar to club root. He attributed the tumors to *Bacterium tumefaciens*.

[3] Black, L. M. A virus tumor disease of plants. Amer. Jour. Bot. 32: 408–415. 1945.

[4] Since this was written Dr. Black has informed the writer that he has compared the symptoms of the clubroot virus and his wound-tumor virus in Turkish tobacco and while they are not identical they are very similar and may be caused by related viruses.

[5] Trotter, A. Sulla presenza di tumori radicali nelle coltivazioni di Tabacco di pieno campo. (On the presence of root tumors in tobacco plantations in the open field.) Ric. Ossvz. Divulg. fitopat. Campania ed Mezzogiorno (Portici) 10: 65–80. 1946. Abstr. in Rev. Appl. Myc. 26: 34. 1947.

A NEW ANTHRACNOSE ON MELONS[1]

DESMOND DOLAN[2]

INTRODUCTION

While breeding muskmelons and watermelons at Spencerport, New York, in 1943, a severe and undescribed disease was found on both muskmelon and watermelon vines. Since 1943, it has become more prevalent, and in both 1944 and 1945 the melon crop of western New York was severely affected by this disease.

SYMPTOMATOLOGY

The disease appears as small pink to light brown spots which coalesce to form long streaks on the petioles and vines. It seldom attacks the laminae of leaves and cotyledons. Diseased petioles become very brittle and break or turn brown and wither so that only the vine remains. The leaves wither and die mostly as a result of petiole infection, but occasionally streaks may be seen on the veins on the under side of the leaf. On the vines, the streaks may attain a length of 7 to 8 inches (Fig. 1). The terminal parts of such vines usually turn brown and die. In humid weather, the pink spore mass may be seen as a film on the disease lesions; but in dry weather, this character is absent.

DESCRIPTION OF THE FUNGUS ISOLATED FROM DISEASED MELONS

Isolations from streaks on watermelon and muskmelon vines always yielded the same fungus. On potato-dextrose agar, the fungus produces a pink growth which spreads rapidly over the surface of the agar. The culture remains pink and does not turn black with age. The surface of the culture is smooth except for radial corrugations which are frequently most pronounced at the margin of the colony (Fig. 2). Pink spore slime is produced in great abundance. The spores are relatively small (8–$10\,\mu \times 1.7$–$3.3\,\mu$), 2-celled, and sub-rectangular.

In Petri-dish cultures from one to many spores are borne in heads on the ends of short conidiophores (Fig. 3, E and G). The head is not surrounded by a membrane. This was evidenced by the fact that (a) heads removed and placed in water disintegrated and the spores were immediately set free, (b) a very dense suspension of spores could be made by running water over the surface of the culture and (c) groups of spores could be seen lying on the surface of the agar as though they had been budded from a single conidiophore without sticking together to form a head (Fig. 3, F). Sometimes the

heads were spherical, possibly because of the presence of a film of water around the spore head.

Ten single-spore cultures were obtained by pouring a very dilute suspension of spores over the surface of solidified agar in a Petri dish, marking isolated single spores and removing them with a "biscuit cutter." Six of

FIG. 1. Muskmelon plants 3 days after inoculation with *Marssonina melonis* n. sp. Note streaks on stems, petioles and veins of cotyledons and leaves. Also note absence of spots in laminae of leaves and cotyledons.

the single-spore cultures were similar to the original culture, but three produced white growth on potato-dextrose agar. The spores of the nine pink isolates had the same dimensions as the spores of the original culture, but the spores of the white isolates were slightly smaller, measuring $5–7 \mu \times 1.5–3.0 \mu$.

PATHOGENICITY OF THE FUNGUS

An attempt was made to induce the disease artificially by inoculating six-week-old Bender muskmelon plants in the greenhouse in December with both the pink and white isolates. The plants were sprayed with a suspension of spores and then placed in a chamber with high humidity and a temperature of 75° F., but no infection resulted. Nongerminated spores were found in large numbers on the leaves 48 hours after inoculation. When 1 gm. of dextrose sugar was added to each 200 ml. of spore suspension before inoculation, abundant infection resulted and typical symptoms of the disease were produced. The disease symptoms developed on the petioles of the cotyledons 40 hours after inoculation. Sixty hours after inoculation, pink spots could be seen on the leaf petioles and on the vines, and these coalesced to form streaks that first became obvious 72 hours after inoculation. It was observed that petioles with pink streaks were very brittle and broke easily.
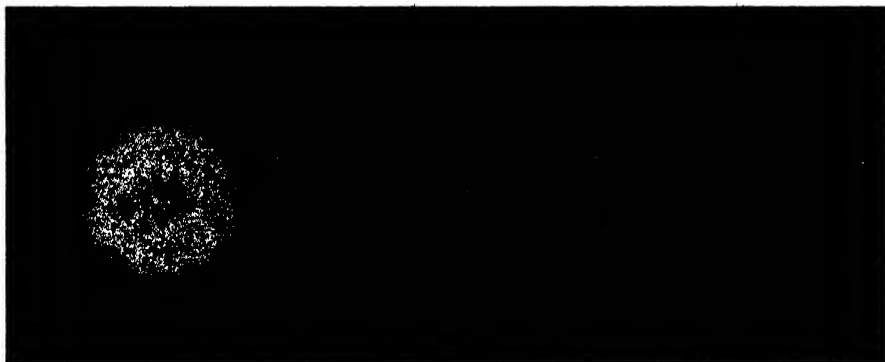
FIG. 2. Cultures of *Colletotrichum lagenarium* Ells. and Hals. (left), pink *Marssonina* (center) and white *Marssonina* (right) after 43 days on potato-dextrose agar at 48.2° F.

Later, the infected petioles collapsed and withered, producing a general plant appearance very similar to that seen in the field. The disease symptoms produced by the pink and white isolates were identical.

HISTOLOGY OF THE DISEASE

The mycelium of *Marssonina* was intracellular (Fig. 3, A). This cross section of a disease streak shows a continuous fruiting layer from which the spores are produced in great abundance. No definite limits of the ascervuli were observed and it was concluded that when ascervuli are formed, they are probably gregarious, running together to form a continuous fruiting layer over the surface of the diseased tissue. At the border between the diseased and healthy tissue, the fruiting layer gradually merges into healthy tissue. No setae were observed protruding from the fruiting layer or at the line of demarcation between diseased and healthy tissue. The stroma is poorly developed and superficial, composed of loosely intertwined hyphae.
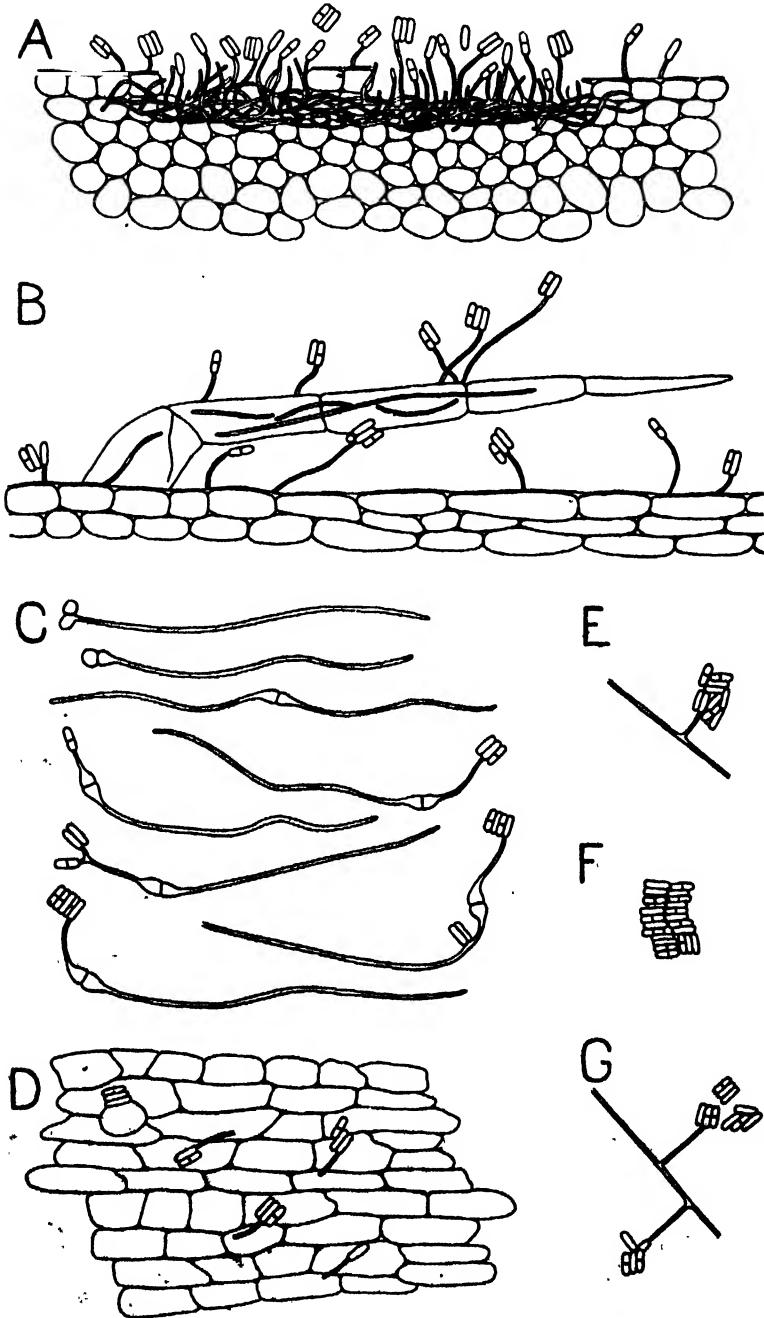
FIG. 3. A. Cross-section of a streak lesion. B. Longitudinal section of streak lesion showing a trichome. C. Germinating spores of *Marssonina melonis* n. sp. D. Vertical aspect of a streak lesion showing conidiophores and spores on surface of leaf. E, F, and G. Spores being produced from conidiophores on the surface of potato-dextrose agar in a Petri dish. All 350×.

Marssonina melonis n. sp.

Maculae parvae, rubicundulae vel pallide fuscae, coalescentes et lineas rubicundulas vel subfuscas vetustate atrofuscas formantes; ascervuli indefiniti, gregarii, struem fructificantem continuam, exiliter evolutam, superficialem, ex hyphis solute implectis compositam formantes; conidia subrectangulata, 8–10 $\mu \times$ 1.7–3.3 μ, medio 1-septata, protoplasmate hyalino.

Habitat: Parasitic on vines, petioles, and leaf veins of *Cucumis melo* var. *reticulatus* and *Citrullus vulgaris* in Munroe and Ontario Counties, New York.

Cultures and photographs have been deposited with the Department of Plant Pathology, Cornell University. Cultures were also sent to Professor David H. Linder, Herbarium of Cryptogamic Botany, Harvard University.

PHYSIOLOGY OF MARSSONINA MELONIS

As mentioned previously, the difficulty in getting infection with *Marssonina melonis* during the winter in the greenhouse could be overcome by adding a small amount of sugar to the spore suspension before inoculation. Consequently, an experiment was planned to determine the effect of time of year and addition of sugar to the spore suspension on the amount of infection with two isolates (one pink and one white) of *Marssonina melonis;* and *Colletotrichum lagenarium* Ells. and Hals. was included as a standard for comparison.

Seeds of the Bender Surprise muskmelon were planted on four different dates: January 15, February 12, March 12, and April 16. The seeds were sprouted in paper towels, and 30 sprouted seeds were planted in each of 6 flats on the above dates. The plants were inoculated at the age of five weeks, two flats being inoculated with each of the three organisms. One of the two flats was sprayed with 200 ml. of a spore suspension to which no sugar had been added. The other flat was sprayed with 200 ml. of a spore suspension to which one gm. dextrose had been added.

The spore suspensions were prepared by washing spores from the surface of two-week-old Petri-dish cultures. The crude spore suspension was then centrifuged at 3000 r.p.m. for 5 min., the supernatant liquid was poured off and the original volume restored by adding distilled water. This was repeated three times until it was considered the spores were washed free of all nutrients that might have been carried over from the medium. After the final washing, the concentration of spores was adjusted to 100,000 per ml. which was equivalent to 50 spores per low power field using a 25× ocular.

After inoculation, the flats were placed in an infection chamber with high humidity and a temperature of 70–75° F. for 48 hours. Eight to ten days, after inoculation, the degree of infection was measured according to a rating system: 0 meaning no infection, 1–3 meaning slight infection, 4–6 meaning medium infection, and 7–9 meaning severe infection. Each plant in the flat was rated and the 30 figures for each flat were averaged to give the degree of infection for each inoculation treatment.

The severity of infection for each organism for each date of planting, with and without the addition of sugar to the inoculum, is recorded in table 1.

Without the addition of sugar to the suspension of spores before inoculation the infection with both isolates of *Marssonina* was slight in the winter months when light conditions were poor. *Colletotrichum*, on the other hand, gave good infection in the winter months without the addition of sugar to the inoculum.

Examination of the leaves after inoculation revealed that lack of infection by the *Marssonina* isolates was due to nongermination of the spores.

TABLE 1.—*Average degree of infection with three organisms at different dates, when inoculated with and without the addition of sugar to the spore suspension before inoculation*

Planting date	Inoculation date	Organism	Average degree of infection	
			Without dextrose	With dextrose
Jan. 15	Feb. 19	*Marssonina* (pink)	0.0	7.3
		do (white)	0.0	7.8
		Colletotrichum	6.4	8.5
Feb. 12	Mar. 19	*Marssonina* (pink)	1.5	6.9
		do (white)	1.8	7.7
		Colletotrichum	5.9	8.7
Mar. 12	Apr. 16	*Marssonina* (pink)	6.5	6.6
		do (white)	6.8	8.0
		Colletotrichum	7.3	7.4
Apr. 16	May 21	*Marssonina* (pink)	7.8	8.1
		do (white)	8.0	8.5
		Colletotrichum	8.9	8.7

When there was no infection with *Marssonina*, nongerminated spores were still seen in abundance on the leaf 48 hours after inoculation.

SPORE GERMINATION TESTS IN VARIOUS NUTRIENT MEDIA

Spore germination studies were carried on to determine (a) the minimum amount of dextrose needed for germination of *Marssonina* spores, (b) to determine if *Marssonina* spores would germinate without sugar in a mineral nutrient solution, (c) to determine if a higher percentage of *Marssonina* spores would germinate in a guttated fluid from melon plants growing in the light than from melon plants growing in the dark.

The germination tests were carried on in clean pyrex Petri dishes. Each Petri dish contained 10 ml. of solution made up as follows: 1 ml. of each nutrient under test, 5 ml. of spore suspension, and the remaining volume of redistilled water.

As a standard of comparison, spores of *Colletotrichum lagenarium* Ells. and Hals. were subjected to the same germination tests as those of the two *Marssonina* isolates. The suspension of spores of each organism was pre-

pared as follows: Spores were washed from the surface of two-week-old
Petri-dish cultures. The suspension was then centrifuged at 3000 r.p.m. for
5 min., the supernatant liquid poured off, and the spores again suspended in
redistilled water. The spores were given three such washings before being
used in germination tests. After the final washing, the concentration of the
suspension was adjusted to 200,000 spores per ml., which is equivalent to
100 spores per low power field using a 25× ocular. Five ml. of this suspen-
sion were placed in each Petri dish.

Germination counts were made on five low power fields using a 25× ocular
at the end of a 12- to 24-hour period, and the average percentage of germina-
tion was recorded.

The average percentage germination of the three isolates in the various
liquid media is recorded in table 2.

TABLE 2.—*Average percentage germination of spores of three organisms in various
liquid media*

Medium	Marssonina (pink)	Marssonina (white)	Colletotrichum
Redistilled water	0.0	0.0	0.6
Distilled water	0.0	0.0	0.0
Tap water	0.0	0.0	3.5
Dextrose in redistilled water			
0.001 mg. per ml.	0.0	0.0	0.8
0.005 do	0.0	0.0	2.2
0.01 do	0.0	0.0	4.5
0.05 do	0.0	0.0	6.6
0.1 do	3.2	2.0	9.1
0.5 do	8.4	11.2	43.3
1.0 do	39.5	29.9	93.1
5.0 do	68.0	31.2	95.2
10.0 do	72.6	65.1	95.4
KNO$_3$ 0.505 gms. per ml. (5 mlmol./liter) KH$_2$PO$_4$ 0.372 gms. per ml. (2 mlmol./liter) MgSO$_4$ 0.120 gms. per ml. (1 mlmol./liter) in redistilled water	0.0	0.0	0.0
Above 5 mg. dextrose per ml. in redistilled water	96.3	97.5	98.0
Guttation from melon leaves in light	63.8	71.2	96.6
Guttation from melon leaves in dark	0.0	0.0	2.2

These results show that the percentage germination of spores of both
Marssonina and *Colletotrichum* increases as the sugar concentration is in-
creased within certain limits. However, spores of *Colletotrichum* germinated
at a lower sugar concentration than those of *Marssonina*.

A dextrose concentration of 5.0 mg. per ml. is approximately the threshold
of germination for *Marssonina* spores. Melon plants growing in abundant
light secrete an energy source in the guttation fluid which is apparently
absent in guttated fluid from plants growing in the dark. Artificial infec-
tion is unsuccessful unless the spores are supplied with an energy source
either naturally from guttation water or artificially by adding sugar to the
spore suspension before inoculation. To insure infection of melon plants

growing in the greenhouse in winter with deficient light, it is necessary to add 5.0 mg. of dextrose to each ml. of spore suspension before inoculation.

THE MANNER OF SPORE GERMINATION

A study of the manner of spore germination was undertaken as follows: Sterile pyrex Petri dishes with moist filter paper in the bottom were used as moist chambers for studying the germination of spores on microscope slides which had been previously dipped in 0.5 per cent dextrose solution.

Drawings of spore germination were made as shown in figure 3, C. It was observed that at the time of germination, the spores were very plainly 2-celled. They germinated from one or both ends; more frequently from both ends. In some cases, a conidiophore arose at one end of the spore and a germ tube at the other. The conidiophore budded off conidia in rapid succession so that they lay side by side as shown in figure 3, C.

EFFECT OF HUMIDITY ON SPORE GERMINATION

Studies were conducted to determine the effect of a range of relative humidities on germination of spores of three organisms: (Mp) the pink

TABLE 3.—*Percentage germination of spores of three organisms in five relative humidities at the end of 24 hours*

Salt	Gm. in 200 ml.	Percentage relative humidity	Percentage germination at end of 24 hours		
			Mp	Mw	C
No salt: H_2O only	......	100.0	100.0	100.0	100.0
$Na_2HPO_4 \cdot 12H_2O$	40	95.0	100.0	100.0	83.1
K_2CrO_4	120	88.0	18.4	27.3	0.0
NH_4Cl	75	79.5	0.0	0.0	0.0
$NaNO_2$	190	69.0	0.0	0.0	0.0

Marssonina, (Mw) the white *Marssonina,* and (C) *Colletotrichum lagenarium* Ells. and Hals.

Medium-size bell jars (diam. 7 inches) were used as humidity chambers. Each bell jar was placed over a Stender dish containing a saturated salt solution to maintain the desired humidity. At their bases, the bell jars were sealed to glass plates with vaseline so as to make them air tight. The salt solutions used and the humidities maintained in the different chambers are recorded in table 3.

All chambers were kept at room temperature. The spores were germinated on microscope slides supported on glass rods above salt solutions. ·

Two hundred ml. of washed spore suspension were prepared as described previously and adjusted to a concentration of 200,000 spores per ml. One gram of sugar was added. Five ml. of the sugar solution spore suspension thus prepared were placed in a small test tube. One ml. KNO_3 solution (50 millimols per liter), 1 ml. KH_2PO_4 (20 millimols per liter), 1 ml. $MgSO_4$ (10 millimols per liter) and 2 ml. of redistilled water were added.

Each of the three nutrient solution spore suspensions were then stirred and smeared with a wire loop on ten clean microscope slides. The slides were then dried in front of an electric fan and two of the slides were placed in each humidity chamber.

Counts of the percentage germination were made at the end of 24 hours (Table 3). Three low power fields were counted on each slide, and the average percentage germination recorded.

Spores of the two *Marssonina* isolates germinated at a lower humidity than spores of *Colletotrichum lagenarium* Ells. and Hals.

EFFECT OF TEMPERATURE ON GROWTH

Experiments were conducted to determine the effect of temperature on the growth of the two isolates of *Marssonina* as compared to *Colletotrichum lagenarium* Ells. and Hals. Sterile potato-dextrose agar was poured into 36 sterile Petri dishes so as to give a uniform depth of medium, and the agar

TABLE 4.—*Diameters in millimeters of colonies of three organisms* (*Mp* = *pink Marssonina, Mw* = *white Marssonina, and C* = *Colletotrichum lagenarium Ells. and Hals.*) *at various time intervals at four temperatures*

Age of culture (Days)	Temperature											
	48.2° F.			59.0° F.			67.1° F.			75.2° F.		
	Mp	Mw	C	Mp	Mw	C	Mp	Mw	C	Mp	Mw	C
3	0	0	0	0	0	0	14	17	4	28	30	29
6	0	0	0	3	5	0	15	18	7	31	40	35
9	6	4	0	18	20	5	24	36	18	52	53	48
12	9	9	2	25	27	15	40	47	36	73	76	66
15	12	13	4	31	32	25	64	66	58	Discarded		
22	20	19	5	57	61	38	Discarded					
29	31	32	6	Discarded								
36	39	43	7									
43	44	49	8									
49	51	68	10									

was allowed to solidify. Twelve Petri dishes were inoculated with each of the three organisms by placing a loop of spore suspension in the center of each plate. Three plates inoculated with each organism were then placed at each of the following temperatures: 48.2°, 59.0°, 67.1°, and 75.2° F.

The diameters of the colonies were measured in millimeters at intervals of 3 days for 15 days, and after that time, at intervals of one week. The average diameters of the three replicates of each isolate at each temperature are recorded in table 4.

At low temperatures, the *Marssonina* isolates grew much more rapidly than the *Colletotrichum* isolate. The white *Marssonina* grows slightly more rapidly than the pink *Marssonina* at low temperatures. At the higher temperatures, the *Marssonina* isolates grew only slightly more rapidly than the *Colletotrichum* isolate. All three organisms displayed greatly increased growth rate with rising temperature and this trend was maintained to 75° F., the highest temperature under test.

TESTING MUSKMELONS AND WATERMELONS FOR RESISTANCE TO
MARSSONINA (PINK ISOLATE) AND COLLETOTRICHUM
LAGENARIUM ELLS. AND HALS.

At the time the study was undertaken, two forms of anthracnose were prevalent in New York State. One form, lately discovered and described in this paper, was caused by an undescribed species of *Marssonina* (Fig. 1). The other form was the regular anthracnose caused by *Colletotrichum lagenarium* Ells. and Hals. (Fig. 4).

FIG. 4.   Leaves, cotyledons, and stems of muskmelon plants 5 days after inoculation with *Colletotrichum lagenarium* Ells. and Hals.

In muskmelons, no resistance to either organism had been discovered. In watermelons, the McCrea resistant variety was reputed to be resistant to the regular anthracnose. With the hope of finding resistance to one or both of these diseases, muskmelons and watermelons from various sources were tested by artificial inoculation with both organisms in the greenhouse.

Forty seeds of each seed lot to be tested were germinated in moist paper towels in an incubator at 25° C. Eight sprouted seeds of each lot were planted in each of four 6-inch pots. One pot was allotted to each of four replicates in which arrangements were at random.

Twenty-five to thirty days after planting, two of the four replicates were inoculated with each of the two organisms, *Marssonina* (pink form) and *Colletotrichum*, by spraying with suspensions of spores of the organisms. The suspensions were prepared by washing spores from the surface of 2-week-

old Petri-dish cultures. The concentration of spores was adjusted to 50 per low power field using a 25× ocular. In order to insure infection with *Marssonina*, one gram of dextrose was added to each 200 ml. of spore suspension. After inoculation, the plants were placed in an infection chamber with high humidity; those inoculated with *Marssonina* for 40 hours and those inoculated with *Colletotrichum* for 48 hours.

TABLE 5.—*Average degree of infection of melons with Marssonina (pink isolate) and Colletotrichum lagenarium Ells. and Hals. after inoculation in the greenhouse*

Variety	Source	Average degree of infection[a]			
		Marssonina		Colletotrichum	
		Planting			
		1	2	1	2
Muskmelons					
Conomon 43–192–n	Cornell University	0.4	2.9	3.6	5.1
Box No. 1—140666	F.P.I.[b]	1.2	4.9	6.8	7.6
Box No. 1—140883	do	5.5	8.3	7.5	8.0
Box No. 2—123493	do	2.0	5.2	6.1	7.0
Box No. 3—125919	do	3.9	8.7	6.1	8.4
Box No. 6—126114	do	6.5	7.7	7.8	8.2
Box No. 8—125998	do	7.0	7.9	8.0	7.3
Honeydew	Asgrow 37542	4.0	4.4	7.9	8.4
Accession 106	E. G. Anderson, 1945	4.1	4.5	6.4	7.5
Honeydew	California, 1945	4.1	6.7	7.2	7.3
Cassaba melon	Asgrow E 324.1	4.5	6.9	8.1	5.7
Bender Surprise	G.L.F., Ithaca, N. Y.	7.0	8.2	9.0	8.2
White melon, *Cucumis melo* conomon	W. D. Enzie, Geneva, N. Y.		2.5		5.9
Freeman cucumber FC–2	do		2.6		.....
FC–3, Inbred 42–9	do		2.4		6.7
Murdela 2–65	do		4.1		5.9
Okase	do		7.9		6.7
FR 13–6, Inbred 42–11	do .		7.3		6.7
Aristogold 43–47	do		6.7		6.7
F₁ Okase 5–39 × FC–2	do		2.3		.....
F₁ Aristogold 43–47 × FC–8	do		3.0		7.7
F₁ K–3 × FR 13–6	do		5.1		7.8
Watermelons					
McCrea resistant	Modesto, California	8.1	7.9	2.9	5.6
Honey Cream	Robson 345	2.9	3.6	7.8	8.5
Tom Watson	Harris 43		9.0		9.0
Dixie Queen	Harris 294		8.3		7.8
Least difference required for significance		19:1	1.8	1.8	2.5	2.1
		99:1	2.6	2.4	3.6	2.8

[a] 0 = no infection, 1 to 3 = slight infection, 4 to 6 = medium infection, 7 to 9 = severe infection.

[b] Federal Plant Introductions, obtained from W. D. Enzie, Geneva, N. Y.

Notes on degree of infection were taken 3–5 days after inoculation with *Marssonina* and 5–7 days after inoculation with *Colletotrichum*. Each plant in each pot was rated according to the degree of infection: 0 measuring no infection, 1–3 meaning slight infection, 4–6 meaning medium infection, and 7–9 meaning severe infection. The ratings of the plants in each pot were averaged, and the average degree of infection recorded. The average de-

grees of infection in the two replicates inoculated with each organism were again averaged and the least significant difference between means was determined.

Two different plantings were inoculated with each organism. The first planting was made on December 20. Two replicates were inoculated with *Colletotrichum* on January 15, and the final notes were taken on January 21. The other two replicates in the first planting were inoculated with *Marssonina* on January 17, and the final notes were taken on January 21. The

TABLE 6.—*Tabulation of frequency of occurrence of each infection rating in resistant and susceptible parents and in segregating backcross and F_2 progenies inoculated with Colletotrichum and Marssonina*

	No. inc.	Colletotrichum									No. inc.	Marssonina (pink)								
		1	2	3	4	5	6	7	8	9		1	2	3	4	5	6	7	8	9
Resistant parents																				
Conomon 43–192–n	14	1	2	6	3	1	1				16	2	6	7	1					
White melon	16		1	0	5	9	1				13		10	3						
FC–3, Inbred 42–9	15	3	2	1	6	2	1				16	3	5	7	1					
Susceptible parents																				
Mardela 2–65	16	1	0	1	3	7	4				14		2	4	6	2				
F.R. 13–6, Inbred 42–11	16		2	0	3	5	6				14				4		2	7	1	
Aristogold	16						4	8	2	2	16				1	0	5	8	1	1
Backcross progenies																				
1. (W–2 × Mard. 2–65) × Mard. 2–65	36						4	10	13	9	28	1	3	14	7	2	1			
2. (K–3 × F.R. 13–6) × F.R. 13–6	40						7	15	13	5	40		5	3	5	5	13	5	3	1
3. Aristogold 43–47 × FC–3	47					2	5	10	17	13	36	6	0	10	7	8	4	1		
F_2 progenies																				
1. W–2 × Mard. 2–65	45						12	19	9	5	31	4	11	5	8	3				
2. K–3 × F.R. 13–6	52					4	6	12	17	13	36		4	9	9	11	1	1	1	
3. Aristogold 43–47 × FC–3	41					1	6	16	11	7	46	6	14	11	7	1	1	3	2	
4. FC–2 × Arist. 43–5	42						8	9	14	11	38	1	8	15	6	5	1	2		
5. FC–2 × Mard. 46–7	44					2	9	13	15	5	45	1	7	10	14	8	2	2	1	

second planting was made on January 26. Two replicates were inoculated with *Colletotrichum* on February 20, and the final notes were taken on February 25. The other two replicates in the second planting were inoculated with *Marssonina* on February 22, and the final notes were taken on February 26.

Segregating backcross and F_2 progenies from crosses made by Professor W. Enzie at Geneva, New York, in which *Cucumis melo* var. *conomon* had been used as one of the parents, were included in the second planting. The individual plants in these progenies were rated according to degree of infection. The average degree of infection of each progeny was not determined. Instead, the ratings 1 to 9 were used as class values, and the frequencies of occurrence in each class were tabulated.

The average degree of infection with both organisms in both plantings is given in table 5. The frequency of occurrence of each infection rating

in the resistant and susceptible parents and in the segregating backcross and F_2 progenies is recorded in table 6.

In the first planting, muskmelons under test varied in resistance to *Marssonina* from the rather resistant Conomon to the very susceptible Bender, and many of the differences are significant. Two muskmelons from the Geneva collection, namely, 140666 and 123493, were significantly more resistant than other muskmelons tested. Honeydew melons and Accession 106 were significantly more resistant than four muskmelons from the Geneva collection and Bender. The Honey Cream watermelon was very resistant to *Marssonina*, but the McCrea resistant watermelon was very susceptible.

With regards to *Colletotrichum*, the McCrea resistant watermelon was very significantly more resistant than any other muskmelon or watermelon tested. Otherwise, the melons under test did not differ significantly in resistance to *Colletotrichum*.

In the second planting, infection with *Marssonina* was more severe than in the first planting. The Conomon melon, White melon, Freeman cucumber, and all their F_1 progenies, except one, were significantly more resistant than 140666, 123493, and most of the other muskmelons tested. White melon, Freeman cucumber, and one of the F_1 progenies were significantly more resistant than Asgrow's honeydew and Mardela, but Conomon 43-192-n and two of the F_1 progenies were not. Mardela, Asgrow's honeydew, Accession 106, 140666, and 123493 were significantly more resistant than Bender and most of the other muskmelons tested.

The Honey Cream watermelon was very significantly more resistant to *Marssonina* than other watermelons tested. The McCrea resistant watermelon was not significantly more resistant to *Marssonina* than the susceptible commercial varieties.

In table 6, it can be seen that backcross progeny 1 gave a higher proportion of plants resistant to *Marssonina* than backcross progenies 2 and 3. All three progenies had some resistant and some susceptible plants, but no ratios of resistant to susceptible plants are discernible. The F_2 progenies had a lower proportion of susceptible plants than the backcross progenies. However, all classes from high resistance to high susceptibility were represented. Such dispersion in a small population made fitting of the tabulated results to any genetic ratios impossible.

With regards to *Colletotrichum*, Conomon, Cassaba melon, White melon, and, McCrea resistant watermelon were significantly more resistant than most of the other melons tested (Table 5). All plants of the segregating backcross and F_2 progenies had medium to severe infection with *Colletotrichum* (Table 6). Most of the plants in each progeny were severely affected. The proportion varied somewhat, but no definite resistance to *Colletotrichum* is discernible in the progenies.

SUMMARY

A new anthracnose is described on muskmelons and watermelons. The most outstanding symptom is small pink to light brown spots coalescing with

age to form dark brown linear lesions. These streaks are mostly on the leaf petioles and vines. Diseased petioles become brittle and break or turn brown and wither so that only the vine remains. Frequently, the terminal parts of the vines become streaked and die.

The disease is caused by a species of *Marssonina*. Its most prominent features are its pink color in culture and its very abundant production of small, two-celled spores which cling together in groups.

During the winter months in a greenhouse with deficient light, the fungus is noninfectious when a spore suspension in distilled water is used as inoculum. Under such conditions, nongerminated spores are found in abundance on the leaves 48 hours after inoculation. Abundant infection results if one gram of dextrose is added to each 200 ml. of spore suspension before inoculation.

In germination tests, spores do not germinate at dextrose concentration less than 0.1 mg. per ml., and 5.0 mg. per ml. is necessary for abundant germination of spores. Spores of *Marssonina melonis* do not germinate in a solution of mineral salts, which indicates that it is an energy source that is needed, but the highest percentage germination is obtained in a medium containing both dextrose and mineral nutrients. Guttated fluid from melon plants growing in abundant light supports the germination of *Marssonina* spores, but there is no germination of spores in guttated fluid from melon plants growing in the dark.

Germination from both ends of the spores is frequent, and spores germinating in nutrient medium at times produce a germ tube at one end and a conidiophore bearing spores at the other end.

Spores of the two *Marssonina* isolates germinate at a lower humidity than spores of *Colletotrichum lagenarium* Ells. and Hals.

At low temperatures, the *Marssonina* isolates grow much more rapidly than *Colletotrichum lagenarium* Ells. and Hals. as measured by the diameters of Petri-dish cultures in millimeters.

Tests for resistance to *Marssonina* revealed that the Conomon melon and its relatives, White melon and Freeman cucumber, display definite resistance, and this resistance is dominant in F_1 progenies. Asgrow's honeydew, Accession 106, and Mardela also have considerable resistance. Several muskmelons from the Division of Plant Exploration and Introduction of the U. S. Department of Agriculture have more resistance than the commercial variety Bender and many other muskmelons tested. The Honey Cream watermelon is much more resistant than other watermelons tested. The McCrea resistant watermelon has no higher degree of resistance than susceptible commercial varieties.

Tests for resistance to *Colletotrichum* revealed that Conomon melon, Casaba melon, and McCrea resistant watermelon are more resistant than other muskmelons and watermelons tested.

RHODE ISLAND STATE COLLEGE,
KINGSTON, RHODE ISLAND.

PHYTOPATHOLOGICAL NOTE

Fasciation in Russet Burbank Potatoes.[1]—In 1944, while surveying Idaho potato cellars in connection with the Emergency Plant Disease Prevention Project, U. S. Department of Agriculture, the senior writer occasionally found tubers with an odd symptom that he named "stitched end." Similar tubers found by grading crews in several lots of potatoes were subsequently submitted for diagnosis. A number of tubers were collected in 1944 and grown in 1945 and 1946 in plots at the Aberdeen Branch Experiment Station. It was found that the tuber symptoms were perpetuated and that plants having flattened stems were produced.

Fig. 1. Second generation fasciated tubers from the original collection made in 1944. Tuber at lower left, normal for the Russet Burbank variety. Remaining tubers illustrate various degrees of fasciation.

Affected tubers have a common symptom that is expressed to various degrees. This is the fusion of buds on the bud end (Fig. 1). At various intervals across the bud end are constrictions and ridges giving the appearance of a tightly sewed grain bag without ears. In nearly all specimens this symptom at once suggested the name "stitched end." Severely affected tubers tend to become flattened and wide on the bud end, giving a wedge-shaped tuber. The most severely affected tubers are flattened and have cleavages of various depths extending from the bud end toward the stem end,

[1] Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper No. 267.
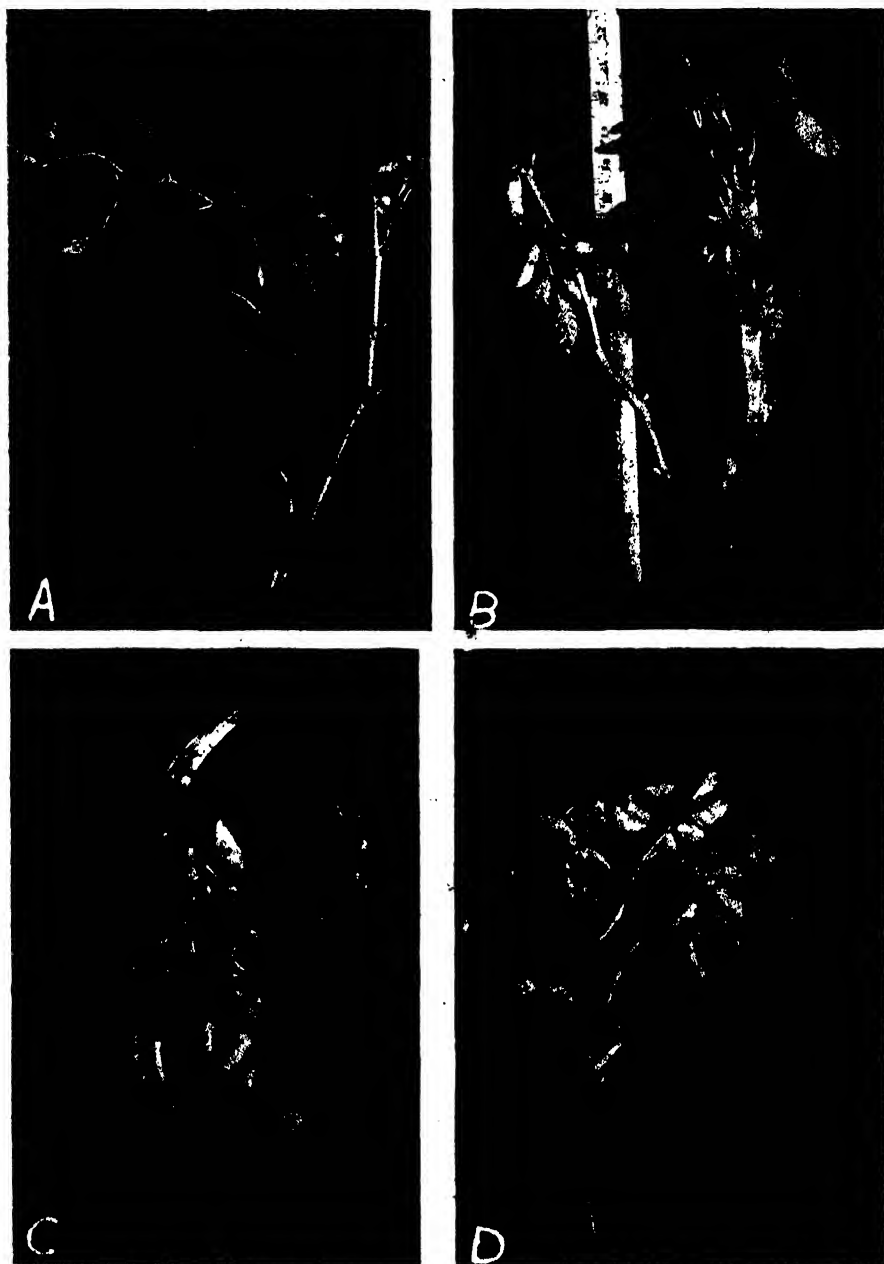
FIG. 2. Fasciation symptoms in the potato plant. A. Base of stem flattened. B, C, and D. Branching, leaf and bud arrangement, and fusion of inflorescences.

suggesting a fusion of tubers. In some cases, because of the extreme distortion, it is difficult to locate the stem end. All tubers in a hill are not equally affected and all gradations of tuber symptoms from apparently normal shaped to the most severely distorted may be present. Eyes on the bud end of affected tubers appear to be small and shallow, and tend to sprout earlier than those on normal tubers.

Plants growing from the affected tubers may also be abnormal. Affected stems are flat or ribbon-like, but all stems from a hill are not fasciated (Fig. 2). Stems an inch or more wide may arise from the same seedpiece as normal-appearing stems. Intermediate stems may be oval shaped in cross section for only a portion of their length. Flattened stems are usually oval shaped at the soil level, but become wider and thinner toward the apex. The terminus of such stems may be curved downward describing some segment of a circle. Completely fasciated stems may be shorter than normal, although some such stems measured 29 inches above the soil surface.

Branching of fasciated stems also varies widely and with no regular pattern. Some completely fasciated stems are devoid of branches. The normal spiral arrangement of leaves and buds is lost, and several flattened nodes may be arranged as to give a whorl-like arrangement of leaves. Fasciated stems have been observed to branch in a dichotomous manner, producing two flattened, nearly equal, stems. In other cases, flattened stems branch to produce one or more laterals which may be either normal or flattened. Often, lateral branches are numerous on the distal half of fasciated stems, many of the branches growing to a greater height than the main axis. Lateral branches frequently are joined to the fasciated axis at an acute angle, and grow nearly parallel to the main axis. Leaves subtending lateral branches are sometimes missing and frequently out of natural position. Petioles may also show evidence of fasciation.

Inflorescences are frequently modified. In extreme cases, all peduncles are fused laterally, leaving only the pedicels and flowers free. When the flowers have abscissed, the free pedicels resemble somewhat the hairs of a brush. This extreme modification of the inflorescence is usually associated with the ribbon-type stem. Normal-appearing branches arising from fasciated stems frequently produce normal inflorescences in contrast to the fused type of the main axis. Flower stalk fusion may involve all stalks of the inflorescence or only two of them. The amount of fusion apparently reflects the extent to which the plant axis is fasciated.

The fasciated condition in potato tubers and plants, so far as known by the writers, is not described in literature. Although the exact nature of this fasciation or its causal factor is not known, it appears to be due to some genetic disturbance that is perpetuated in affected stock. Fasciation in several plant families has been described and attributed to gene mutations or various environmental stimuli.[2] The retention of plant vigor and tuber development by successive tuber generations suggests that this fasciation

[2] White, Orland E. The biology of fasciation and its relation to abnormal growth. Jour. Heredity. 36: 11-22. 1945.

of potatoes is not due to a virus of the degenerative type.   However, there is observational evidence that spindle tuber may mask the symptom of fasciation.

Affected plants and tubers are rare.   The junior writer has found six fasciated plants in commercial fields during the past two years.   It would be difficult or impossible to eliminate all affected tubers from a seed stock, as many tubers carrying the factor do not have symptoms, and seedpieces carrying the factor may not produce fasciated plants.—EARLE C. BLODGETT, formerly Plant Pathologist, Emergency Plant Disease Prevention Project, U. S. Department of Agriculture, Moscow, Idaho, and L. W. NIELSEN, Associate Horticulturist, Idaho Agricultural Experiment Station, Aberdeen, Idaho.

ANNOUNCEMENT

The thirty-ninth annual meeting of The American Phytopathological Society will be held with A. A. A. S. at the Hotel Stevens in Chicago, Illinois, December 28–31, 1947. There will be joint sessions with The Potato Association of America, The Botanical Society of America, and The Mycological Society of America. All meetings will be held in Hotel Stevens.

Abstracts of papers to be presented at the meeting must be in the Office of the Secretary of The American Phytopathological Society by October 15, 1947.

GEORGE GRANT HEDGCOCK

1863–1946

PERLEY SPAULDING

The death of Dr. George Grant Hedgcock on May 11, 1946, made another gap in the thinning ranks of the pioneer plant pathologists of this country.

He was born at Augusta, Illinois, October 5, 1863, one of six children of Barnett and Sarah Lutitia (Haines) Hedgcock. In the general movement westward of many families from the region east of the Mississippi River, the Hedgcocks migrated and settled on a farm at Nora, Nebraska, where George got his early schooling. After five years on the farm he taught public schools from 1882 to 1891, and was principal in the graded schools of Oak and Ruskin, Nebraska, from 1891 to 1895.

He married Laura Ladell Merrill in 1892. She is still living, as are the three children, Mrs. Ruthe Elaine Stevenson, Leland Merrill, and Mrs. Margaret Delletta Church.

He began his college work in 1895, at the University of Nebraska at a considerably more advanced age than most students, taking his B.S. in 1899 and A.M. in 1901. While at the University of Nebraska he had the stimulating training of Charles E. Bessey in laboratory methods and technique in plant pathology, and worked under F. E. Clements on the water relations of plants (2). In 1906 he was given the degree of Ph.D. by Washington University at St. Louis.

It is difficult for younger workers to realize the meagerness in the early 1900's of our knowledge of the fungi causing diseases of plants. Ellis, Peck, and others were making numerous new species, and a great many correctly, according to our present standards. It was distinctly a time of reconnaissance by mycologists, and even more so by pathologists working in our fields and forests. One could expect to find legitimate new species in almost any new field of careful work. In such a time of intense interest for the appreciative mind, Hedgcock began his scientific work.

In his last years at the University of Nebraska he began investigation of sugar-beet diseases, a serious problem with the then young industry in Nebraska (1, 4). He discovered a bacterial disease of beets which was later further investigated by Haven Metcalf (3). In 1901 he was appointed scientific aid in the United States Department of Agriculture and began work at Lincoln, Nebraska, on the sugar-beet diseases. In July, 1902, he was called from Nebraska to St. Louis as assistant in pathology on the staff of the Mississippi Valley Laboratory of the recently organized Bureau of Plant Industry in the United States Department of Agriculture. Here he worked on a number of vegetable diseases, but most intensively on crown gall and hairy root of apple (5) and grape (10). He became convinced that bacteria were the causal agents, but failed to locate the exact tissue
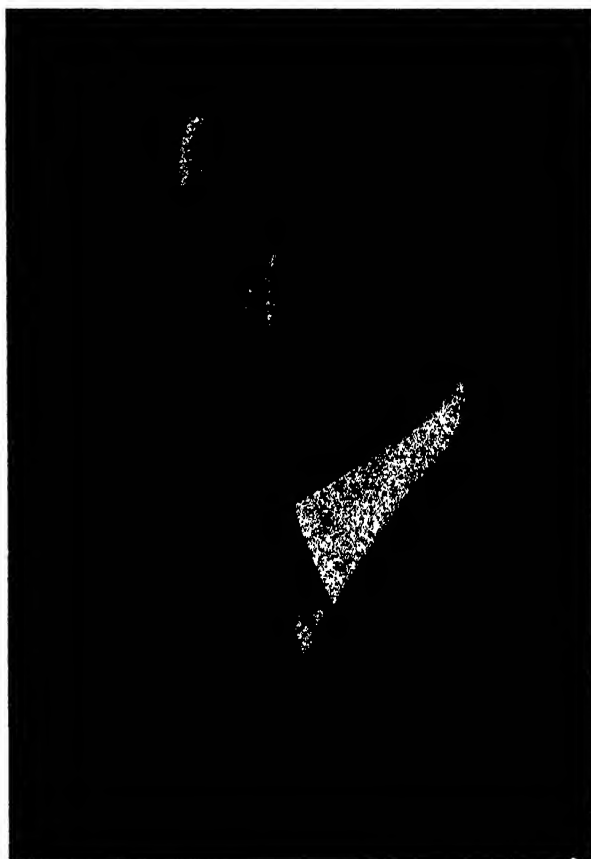
GEORGE GRANT HEDGCOCK
1863–1946
(Photo by Harris and Ewing)

infected. He worked out a practical method of wrapping root grafts at the nurseries, that decidedly reduced the amount of infection (6, 11). Extensive inoculation experiments indicated that crown gall of various plants is intercommunicable and presumably caused by a single organism (8). His field tests showed the disease to be destructive to grape (10), but much less so to the apple (8).

In 1903, he became interested in the fungi causing stain of sapwood in lumber, his first entry into the field of forest pathology. He published the first comprehensive account of these economically important fungi (7). Continuing some tentative experiments by H. von Schrenk, he began attempts to control sap stain by dipping freshly cut lumber in water solutions of chemicals, of which borax and sodium bicarbonate have found some commercial application. Later similar tests were made with basket veneers (12, 43).

In 1907, the Mississippi Valley Laboratory was discontinued and the Office of Investigations in Forest Pathology organized in Washington, D. C., with Haven Metcalf as chief. Hedgcock was transferred to the staff of the new organization with Metcalf and Spaulding. This reoriented the work from general pathology in the Mississippi Valley to strictly forest pathology covering the entire country. He conceived and energetically conducted for a number of years a disease survey of the National Forests. In this he explored as many of the National Forests as means of travel would permit, making copious collections that facilitated laboratory study and permitted distribution of duplicates. On one occasion, after receipt of unusually abundant collections, on his return from the field he was called into his chief's office. There, he was told, with an apparent severity but with a twinkle in the eye, "George, I sent you out to collect fungi, not to eradicate them." A favorite saying of his was, "The time to collect is when collecting is good." Over the years he built up a large study collection, numbered, arranged, and indexed to facilitate ready reference. He also contributed indefatigably to the card index of literature on forest pathology. His early contacts with the personnel of the National Forests helped to pave the way for the permanent location of forest pathologists at several of the Forest Service Regional offices and, somewhat later, at some of the Forest Experiment Stations, to work on critical forest disease problems of their regions. The general results of this survey work were summarized very briefly in two series of papers; one on the trunk rots (14), and the other on the stem and leaf rusts (16). Later, he summarized some of his results in a paper on the fungi found causing diseases in conifers (42) and another on the forest fungi found in the southeastern States (50). Although he collected the destructive mistletoes extensively (18), partially revised them in his collections, and did some cross inoculation work (32), he published little on them.

Early in his disease survey work he became intensely interested in the rusts, which are so generally distributed on the conifers of the entire country. The life history of many was still unknown and presented a fascinat-

ing field for experimentation. This, he began in 1908, and continued over the years with the collaboration of W. H. Long, N. Rex Hunt, G. G. Hahn, and Ellsworth Bethel at various times. The alternate stages of the following rusts were first proved by cross inoculations, usually repeated many times on numerous hosts and through several generations: *Cronartium coleosporioides* (Diet. & Holw.) Arth. (17), *Cr. comandrae* Peck (21), *Coleosporium delicatulum* (Arth. & Kern) Hedgc. & Long (19), *Col. inconspicuum* (Long) Hedgc. & Long (19), *Col. elephantopodis* (Schw.) Thümen (27, 37), *Col. ipomoeae* (Schw.) Burr. (28), *Col. terebinthinaceae* (Schw.) Arth. (29, 31), *Col. helianthi* (Schw.) Arth. (30), *Col. minutum* Hedgc. & Hunt (35), *Col. apocynaceum* Cke. (35).

Rust on pine cones in the southern States was found to be widely distributed and locally plentiful. His studies proved that the alternate hosts were species of *Castanea* and *Quercus* and indicated that there were two of these cone hypertrophying species that were named *Cronartium strobilinum* (Arth.) Hedgc. & Hahn and *Cr. conigenum* (Pat.) Hedgc. & Hunt (36), and were entirely distinct from the stem gall rusts with which they had been confused. A series of papers on the rusts was published in later years on the results of numerous cross inoculations (37); key to aecial stages of *Coleosporium* (39); distribution of the Coleosporiums (44, 47, 48, 51); comparative inoculation data of the known five pine-oak Cronartium species or forms when inoculated onto *Castanea* and related genera (49); comparative cross inoculations of *Cr. cerebrum* Hedgc. & Long and *Cr. fusiforme* Hedgc. & Hunt (22); on *Cr. comandrae* (24, 25); and on *Tuberculina maxima* Rostrup (45). He had a leading part in the important accomplishment of determining that the native pinon blister rust *Cronartium occidentale* Hedgc., Bethel & Hunt is distinct from the introduced *Cr. ribicola* Fischer (34). He was the principal contributor of data to a most useful paper, "Host Relationships of the North American Rusts, other than Gymnosporangiums, Which Attack Conifers" (33).

In a period of reconnaissance work, from which forest pathology has by no means yet fully emerged, he made known numerous serious tree diseases (40). He discovered *Polyporus amarus*, the cause of the brown pocket rot of incense cedar (9), and *Dothichiza populea* Sacc. & Briard, chiefly on Lombardy poplar in this country (26, 38). He called attention to the brown leaf spot of southern pines, a serious growth inhibitor of young seedlings (41). Much work was done on various rots in living forest trees (15, 23), ending with an extensive field study of decays in oaks, yellow poplar, and basswood, which was prepared for publication by G. H. Hepting (46).

He became interested early in the effects of smelter fumes on the vegetation of the vicinity. He learned the characteristic symptoms of the injury to leaves of trees and shrubs as compared with climatic injuries (13, 20), and made extensive collections. Because he was the only available authority on the subject, he was continued on duty by presidential order for three years after reaching retirement age, to assist in the settlement of damage claims against the smelter at Trail, British Columbia.

He continued active field work in the southern states for a number of years after final retirement, and worked over his collections quite regularly until his health failed, in 1941. He published 112 articles and bulletins, of which only the most significant ones are given in the appended list. Hedgcock was a man of great energy, with keen visual perception, and was very enthusiastic in his scientific work. He worked extensively and covered an immense territory, yet he worked intensively culturing the wood-staining fungi and rusts. He had the faculty of making his experimental plants thrive. In fact, his hobby at home was growing flowers.

He was a fellow of the American Association for the Advancement of Science; and member of the American Phytopathological Society, Botanical Society of America, Washington Botanical Society, Mycological Society of America, Cosmos Club, Sigma Xi, Masonic Fraternity, and Presbyterian Church.

SOME OF THE MORE SIGNIFICANT PUBLICATIONS OF WHICH DR. HEDGCOCK WAS AUTHOR OR CO-AUTHOR

1. Nebraska sugar beets. Omaha Trade Exhibit 12: 22. 1901.
2. The relation of the water content of the soil to certain plants, principally mesophytes. Univ. of Nebr. Seminar, Bot. Survey Nebr. 6, 79 pp. 1902.
3. Eine durch Bakterien verursachte Zuckerrübenkrankheit. Ztschr. Pflanzenkr. 12: 321–324. 1902. (With HAVEN METCALF.)
4. Proof of the identity of Phoma and Phyllosticta on the sugar beet. Jour. Mycol. 10: 2–3. 1904.
5. The crown-gall and hairy-root diseases of the apple tree. U. S. Dept. Agr., Bur. Plant Indus. Bul. 90(2): 1–7. 1905.
6. The wrapping of apple grafts and its relation to the crown-gall disease. U. S. Dept. Agr., Bur. Plant Indus. Bul. 100(2): 1–12. 1906. (With HERMANN VON SCHRENK.)
7. Studies upon some chromogenic fungi which discolor wood. Mo. Bot. Gard. Rpt. 17: 59–114. 1906.
8. The cross-inoculation of fruit trees and shrubs with crown-gall. U. S. Dept. Agr., Bur. Plant Indus. Bul. 131(3): 21–23. 1908.
9. A new polypore on incense cedar. Mycologia 2: 155–156. 1910.
10. Field studies of the crown-gall of the grape. U. S. Dept. Agr., Bur. Plant Indus. Bul. 183. 1910.
11. Field studies of the crown-gall and hairy-root of the apple tree. U. S. Dept. Agr., Bur. Plant Indus. Bul. 186. 1910.
12. Prevention of mould. Barrel and Box 16(4): 35. 1911.
13. Winter-killing and smelter-injury in the forests of Montana. Torreya 12: 25–30. 1912.
14. Notes on some diseases of trees in our National Forests. Phytopath. 2: 73–80. 1912; 3: 111–114. 1913; 4: 181–188. 1914; 5: 175–181. 1915.
15. Preliminary notes on three rots of juniper. Mycologia 4: 109–114. 1912. (With W. H. LONG.)
16. Notes on some western Urodineae which attack forest trees. Mycologia 4: 141–147. 1912; Phytopath. 3: 15–17. 1913.
17. The Cronartium associated with Peridermium filamentosum Peck. Phytopath. 2: 176–177. 1912.
18. Notes on diseases of trees caused by mistletoes. Jour. Washington Acad. Sci. 3: 265–266. 1913.
19. Notes on cultures of three species of Peridermium. Phytopath. 3: 250–251. 1913. (With W. H. LONG.)
20. Injury by smelter smoke in southeastern Tennessee. Jour. Washington Acad. Sci. 4: 70–71. 1914.
21. The alternate stage of Peridermium pyriforme.- 3 pp. 1914. Privately printed. (With W. H. LONG.)
22. Identity of Peridermium fusiforme with Peridermium cerebrum. Jour. Agr. Res. [U.S.] 2: 247–249. 1914. (With W. H. LONG.)

23. Heart-rot of oaks and poplars caused by Polyporus dryophilus. Jour. Agr. Res. [U.S.] 3: 65–78. 1914. (With W. H. LONG.)
24. A disease of pines caused by Cronartium pyriforme. U. S. Dept. Agr. Bul. 247. 1915. (With W. H. LONG.)
25. Two new hosts for Peridermium pyriforme. Jour. Agr. Res. [U.S.] 5: 289–290. 1915. (With W. H. LONG.)
26. Dothichiza populea in the United States. Mycologia 8: 300–308. 1916. (With N. R. HUNT.)
27. The aecial stage of Coleosporium elephantopodis. Phytopath. 7: 66–67. 1917. (With W. H. LONG.)
28. The Peridermium belonging to Coleosporium ipomoeae. Phytopath. 7: 67. 1917. (With N. R. HUNT.)
29. A Peridermium belonging to Coleosporium terebinthinaceae. Phytopath. 7: 67. 1917. (With N. R. HUNT.)
30. An alternate form for Coleosporium helianthi. Phytopath. 7: 67–68. 1917. (With N. R. HUNT.)
31. New species of Peridermium. Mycologia 9: 239–242. 1917. (With N. R. HUNT.)
32. Notes on Razoumofskya campylopoda. Phytopath. 7: 315–316. 1917. (With N. R. HUNT.)
33. Host relationships of the North American rusts, other than Gymnosporangiums, which attack conifers. Phytopath. 8: 309–352. 1918. (With A. S. RHOADS, ELLS-WORTH BETHEL, and CARL HARTLEY.)
34. Pinon blister-rust. Jour. Agr. Res. [U.S.] 14: 411–424. 1918. (With ELLSWORTH BETHEL and N. R. HUNT.)
35. New species and relationships in the genus Coleosporium. Mycologia 12: 182–198. 1920. (With N. R. HUNT and G. G. HAHN.)
36. Two important pine cone rusts and their new cronartial stages. Part I. Cronartium strobilinum (Arthur) Hedgc. and Hahn, comb. nov. Phytopath. 12: 109–116. 1922. (With G. G. HAHN.) Part II. Cronartium conigenum (Pat.) Hedgc. and Hunt, comb. nov. Phytopath. 12: 116–122. 1922. (With N. R. HUNT.)
37. Notes on some species of Coleosporium. Mycologia 14: 244–257. 1922; 14: 297–310. 1922; 25: 392–396. 1933. (With N. R. HUNT.)
38. Dothichiza populea and its mode of infection. Phytopath. 17: 545–547. 1927.
39. A key to the known aecial forms of Coleosporium occurring in the United States and a list of the host species. Mycologia 20: 97–100. 1928.
40. The large leaf spot of chestnut and oak associated with Monochaetia desmazierii. Mycologia 21: 324–325. 1929.
41. Septoria acicola and the brown-spot disease of pine needles. Phytopath. 19: 993–999. 1929.
42. Notes on the distribution of some fungi associated with diseases of conifers. U. S. Dept. Agr., Plant Dis. Rptr. 16: 28–42. 1932.
43. The prevention of wood-staining in basket veneers. Jour. Forestry 31: 416–420. 1933.
44. Notes on the distribution of some species of Coleosporium in the United States and adjacent regions. U. S. Dept. Agr., Plant Dis. Rptr. 17: 20–27. 1933.
45. Notes on the occurrence of Tuberculina maxima on the aecia of Cronartium cerebrum. Phytopath. 25: 1117–1118. 1935.
46. Decay in merchantable oak, yellow poplar, and basswood in the Appalachian Region. U. S. Dept. Agr. Tech. Bul. 570. 1937. (With G. H. HEPTING.)
47. Notes on the occurrence of Coleosporium crowellii Cummins in the United States. U. S. Dept. Agr., Plant Dis. Rptr. 23: 133–134. 1939.
48. Notes on the occurrence of Coleosporium in the southeastern United States during 1938 and 1939. U. S. Dept. Agr., Plant Dis. Rptr. 23: 268–277. 1939.
49. Notes on North American pine-oak species of Cronartium on Castanea, Castanopsis, and Lithocarpus. Phytopath. 29: 998–1000. 1939.
50. Notes on the distribution of fungi collected in the southeastern United States in 1938 and 1939. U. S. Dept. Agr., Plant Dis. Rptr. 24: 320–325. 1940.
51. Notes on Coleosporium jonesii (Pk.) Arthur. U. S. Dept. Agr., Plant Dis. Rptr. 25: 244–249. 1941.

ALTERNARIA LEAF BLIGHT OF HEVEA RUBBER TREES

W. J. MARTIN[1]

An undescribed leaf blight of *Hevea brasiliensis* (H.B.K.) Muell. Arg. was observed in a budwood garden on the Campo Experimental de Hule at El Palmar, Veracruz, Mexico, in early April, 1946. Considerable defoliation was occurring in a plot of clone GA–1279. Several other clones in the garden were not affected by the disease and the blight was of little or no importance on any of the 135 other clones on the station. Relatively little infection was observed in *Hevea* seedling nurseries; however, an occasional plant was defoliated.

SYMPTOMS

When young leaves of GA–1279 are heavily infected, partial or entire defoliation occurs early in the development of the leaf cycle. Such leaves have a scorched appearance. Infected leaves which develop to maturity may have brown, concentrically zonate spots in between the veins of the leaf, or somewhat elongate spots along the main veins. Many of the infected leaves which reach maturity have dead distal portions. Leaves as heavily infected as those shown in figure 1 ordinarily are lost before they mature.

ETIOLOGY

An *Alternaria* was commonly associated with the blight, and inoculation experiments were made with spores from diseased leaves and later with spores from pure cultures of the fungus. Typical blight symptoms developed on young leaves of clone GA–1279 within 6 days after artificial inoculations were made, and defoliation occurred on most of the inoculated plants. Leaves up to 3 or 4 days old were the most susceptible; leaves older than 10 days did not become infected even though injured by pricking with a needle.

A search for possible other hosts of the fungus was made in the vicinity of the infected gardens, but none were found.

The Fungus. No records of the occurrence of parasitic species of *Alternaria* on *Hevea* were found. Although detailed comparative studies of the fungus have not been made, it does not seem to fit into any of the species of *Alternaria* described by Groves and Skolko[2] and by Neergaard.[3]

The following observations on the fungus have been recorded. Sporulation on the host is moderate to abundant; the conidia are formed either singly or in short chains. The conidiophores are $50–200 \times 4–6$ μ. The conidia are $50–106 \times 10–17$ μ and have 3–9 transverse septa and 0–3 longitudinal septa.
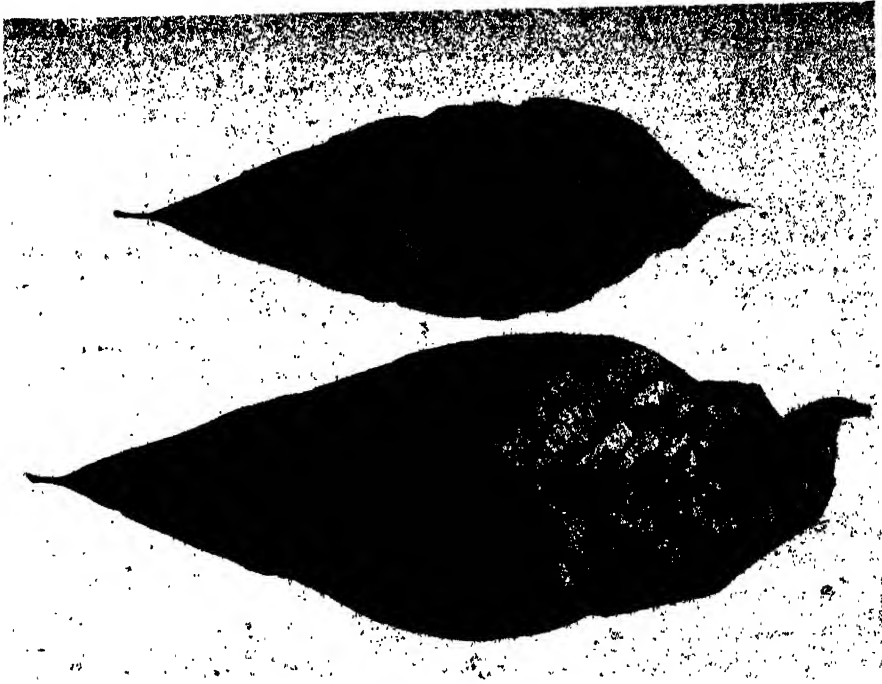
FIG. 1. *Alternaria* leaf blight of *Hevea brasiliensis.* Two naturally infected leaflets of clone GA–1279, which were dropped before reaching maturity. ×2/3 approx.

The fungus was readily cultured on potato-dextrose agar on which it produced a grayish, fluffy, mycelial growth, but in our tests sporulation did not

CONIDIOPHORES
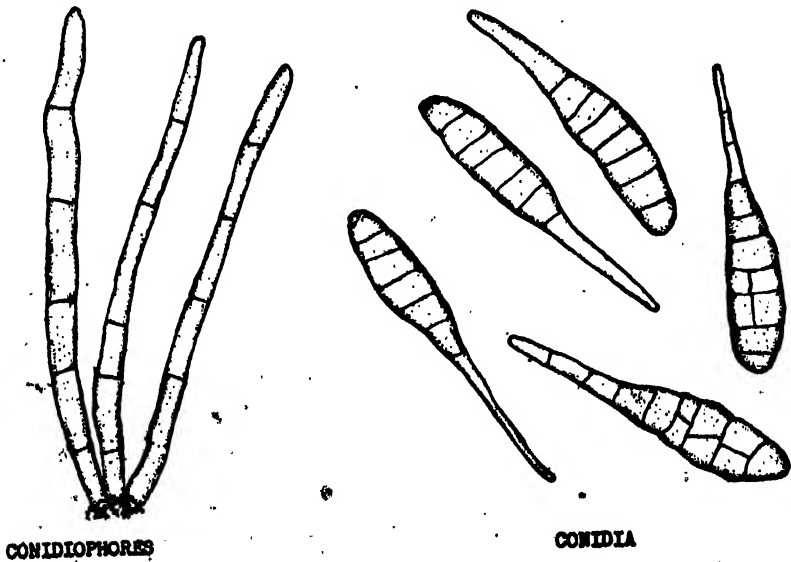
CONIDIA

FIG. 2. Conidiophores and conidia of *Alternaria sp.* on *Hevea brasiliensis.* x 380.

occur on this medium even after 4 weeks. When mycelium from potato-dextrose agar was transferred to plain water agar a few spores were produced within 5 to 10 days. Conidiophores and conidia from *Hevea* leaves are shown in figure 2.

A HOMOPTERA IN RELATION TO THE BLIGHT

An infestation by a homopterous insect (*Tomaspis inca* Guer.[4]) was observed in the nursery and budwood gardens at El Palmar in June, 1946. At this time the blight was observed spreading into a plot of clone GV–42 which had been uninfected previously even though it was adjacent to the heavily infected plot of GA–1279. An experiment was made to determine the possible relation of this insect to the sudden attack of *Alternaria* blight on clone GV–42. Cheesecloth cages were placed over 8 plants of GA–1279 and 8 plants of GV–42. These plants were selected so that each had new leaves just forming and without evidence of infection. Two caged plants

TABLE 1.—*Disease symptoms on leaf flushes of clones GA-1279 and GV-42 seven days after treatment with Alternaria sp., Tomaspis inca, and a combination of the 2 organisms*

Treatment	Disease symptoms on:	
	GA–1279	GV–42
None—control	None	None
Alternaria alone	Typical blight	None
Insects alone	Insect punctures	Insect punctures
Alternaria plus insects	Typical blight (heavy)	Typical blight (light)

of each clone were given the following treatments: 1. No treatment—control. 2. *Alternaria* spores atomized on young leaves. 3. Ten adult insects (*Tomaspis inca*) added to the cages. 4. Ten adult insects added to the cages and *Alternaria* spores atomized on the young leaves. The insects used in the experiment were obtained from an isolated nursery where *Alternaria* blight was not present. They started feeding on the tender leaves and petioles of the plants soon after they were added to the cages. The results are given in table 1.

Additional inoculation tests proved that GV–42 could be infected by spraying *Alternaria* spores on young leaves previously injured by pricking with a needle. The results given in table 1 show that *Alternaria* can penetrate and parasitize the leaves of clone GA–1279 without the aid of insect or mechanical injury, but insect injury results in more severe symptoms. On the other hand, clone GV–42 apparently is resistant to the *Alternaria* and some injury to the leaf tissues is necessary to insure even light infection. Relatively few leaves were lost in clone GV–42 as a result of insect plus *Alternaria* damage.

Weekly spraying with copper fungicides promised successful control of

[4] Identified by Louise M. Russell, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, Washington, D. C.

the blight in clone GA–1279.   No control measures were deemed necessary in other clones.

SUMMARY

A leaf blight of *Hevea brasiliensis* is described and the causal organism established as a species of *Alternaria*.   The blight was severe only on clone GA–1279; other clones growing at El Palmar, Veracruz, Mexico, were resistant to the blight.   Slight infection developed on clone GV–42 which had been infested with an insect, *Tomaspis inca*.

U. S. DEPARTMENT OF AGRICULTURE.

AN ANATOMICAL STUDY OF CROWN–GALL TUMORS ON THE HIMALAYA GIANT BLACKBERRY (RUBUS PROCERUS)

S. G. JONES

INTRODUCTION

Material of the Himalaya giant blackberry, showing natural infection of the stem with crown-gall disease, proved to be very suitable for a study of the type of gall caused by *Bacterium tumefaciens* on various species of *Rubus*. The organism was easily isolated from the surface of these so-called sub-aerial galls.

It is not intended in this brief account to review the extensive literature on crown gall, nor to trace the development of the galls on inoculated material, as this has been done, on other hosts, by numerous authors (1 through 10).

As certain features concerned in crown-gall formation are revealed with unusual clearness in the Himalaya blackberry, advantage has been taken in the present study to bring these out by means of large-scale drawings which have been compounded from numerous sketches made to scale with the camera lucida.

In these representations of the mature gall structure, the most prominent features, which as far as the writer is aware, have not previously been emphasized, are (a) the part played by certain lignified increments to the medullary rays in the disruption of the woody axis of the host, (b) the formation of an "intrusive parenchyma" in the disrupted axis, and (c) the distribution at various places within the gall of numerous meristematic zones which may all be traced to the pericycle.

A transverse section of the stem of this species of blackberry shows the axial cylinder to consist largely of thick-walled fibers, together with a smaller amount of tracheids and of much wider vessels (Fig. 1). The primary medullary rays are prominent throughout their entire radial length, particularly in the phloem by reason of their strongly lignified tracheids in this tissue. As the parenchyma of the rays passes into the pith the cells increase in size and the bulky medulla consists of two kinds of cells, both with cellulose walls, the one large, with scanty protoplasmic contents, the other small, pitted, and apparently empty, arranged in compact groups regularly distributed amongst the larger parenchyma. The secondary phloem is of the usual structure.

Groups of pericycle fibers, thick-walled and lignified, stand out prominently over the vascular bundles (Figs. 2, 3). But the outer one or two layers of the pericycle, consisting of regular rectangular cells with thin cellulose walls, are immediately noticeable in the preparations as they form a more or less continuous sheath around the entire stele (Fig. 2). The im-

FIG. 1.   Trans. section of stem, showing general plan of host and gall tissues.   Inset, in circle, section of cane showing entire gall in relation to the axis.   The large-scale drawing is taken from right-hand portion, at junction of gall and stem.   (A) the outer layers of the pericycle, becoming meristematic.   (B) the cortical host tissues.   (C) the outer pericyclic cambium fanning out into the gall to form secondary parenchyma (See figure 4). (D) the thick-walled tracheids of medullary ray, added to from above by the pericyclic cambium; note the torn phloem and cavities, and crystals; the deep dent in the surface of the axial cylinder opposite the medullary ray.   (E) displaced vascular bundle.   (F, G) tracheids and fibers, developed probably from medullary ray and cambium, sweeping out into the base of the gall.   (H) intrusive parenchyma. (See figure 5).

portant function of these layers of the pericycle in gall formation has been pointed out by numerous authors.   In the present account these outer cells of the pericycle are called the "outer pericyclic cambium" for it is evident from the preparations that similar cells immediately below the groups of pericycle fibers may also become meristematic during gall formation, and these are referred to here as the "inner pericyclic cambium" (Fig. 3, A). The activities of these two pericyclic cambiums are discussed below.   To complete the description of the stem tissues, there remain to mention the cortical region, consisting of a zone of thin-walled parenchyma of variously
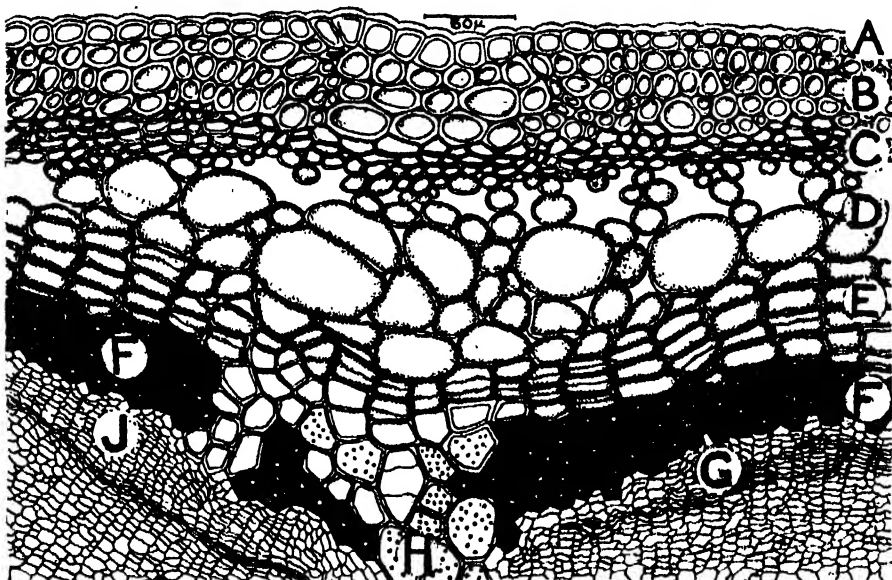
FIG. 2.   Trans. section of portion of stem near a gall, showing the outer tissues, and differentiation of the outer and inner pericyclic cambiums.   (A) cuticle and epidermis. (B) collenchyma, (C) seat of the phellogen, (D) cortex of large and small cells.   (E) the outer pericyclic cambium.   (F) thick-walled fibers of the pericycle.   (G) inner peri-cylic cambium; note zone of crushed, phloem-like tissue below.   (H) the end of a medul-lary ray, with pitted tracheids above, formed from outer pericycle; note similar tracheids amongst pericycle fibers to the left (See figure 4, B).   (J) as for (G).

shaped cells, a feebly active phellogen between it and a more or less continu-ous belt of collenchyma and, finally, the epidermis covered by a thick cuticle (Fig. 2).

GALL DEVELOPMENT

Infection presumably having occurred through a wound (as is always the case in crown-gall infection) in the surface of the stem, the stimulus to gall formation appears to affect first the outer layers of the pericycle so that they become meristematic, and hence are called here the outer pericyclic cambium.   The cells of this cambium, which as already stated surround the entire stele, divide tangentially when they pass over the ends of the medul-lary rays, in the vicinity of gall formation, and the cells formed from them

inwardly are added to the radial length of the medullary rays in the phloem. Most of these new cells are immediately converted into lignified tracheids; sometimes, two or more such cells together become incorporated within a common lignified wall (Fig. 3, B). With the continued formation of these new tracheids, prominent wedges of lignified tissue, having their thin ends impinging on the solid axial cylinder, are developed in the phloem. With the addition of these increments over the ends of the medullary rays, it follows that there is much distortion of the rays themselves with considerable tearing of the delicate tissues of the phloem in the vicinity. These features
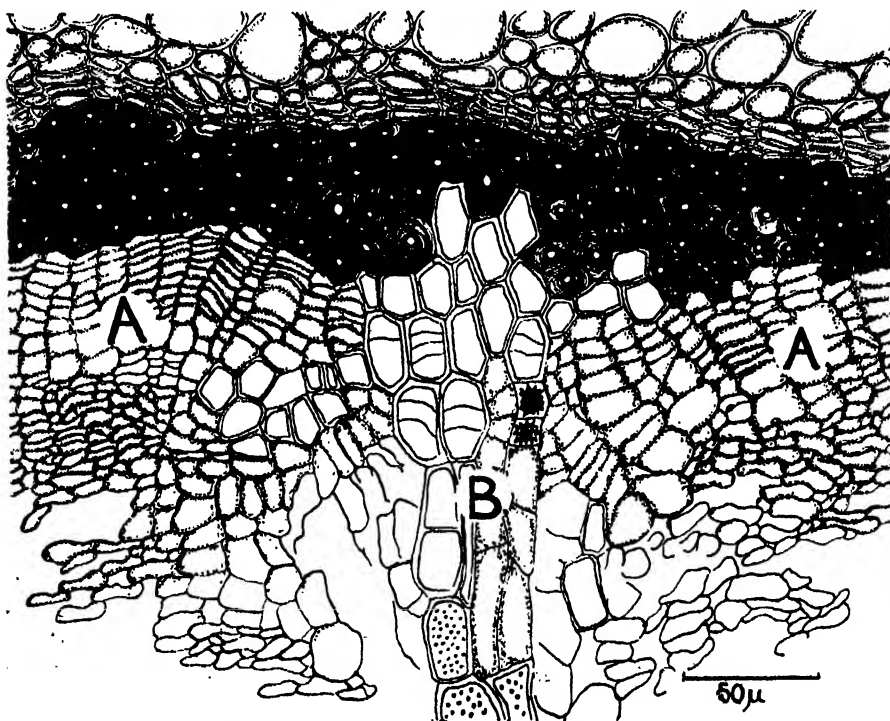
FIG. 3. Trans. section of portion of stem, showing the outer and inner pericyclic cambiums, the latter in active formation of parenchyma to replace the torn phloem below. (A) regular radial arrangement of the new phloem parenchyma. (B) tracheids over the end of a medullary ray, some of which have been formed by the inner pericyclic cambium; note the five tracheids still showing thin cross walls, indicating derivation from a cambium.

are accompanied by much deposit of crystals, probably of calcium oxalate, a similar appearance being also described by Riker (5) in his study of gall on the stem of tomato.

It appears to be the function of the inner pericyclic cambium to compensate for the damage done to the phloem, for the radial rows of parenchyma formed by it are in no way distinguishable from normal phloem. It is, of course, difficult to say whether these innermost cells of the pericycle are actually not those of the protophloem or the phloem itself, but the preparations show that the meristematic cells are close to the fibers, and crushed

protophloem-like cells may be seen at an appreciable distance below (Fig. 2, G).   In some cases there is evidence that the inner pericyclic cambium is capable of forming not only new phloem-like parenchyma, but also lignified tracheids in the same way as the outer pericyclic cambium, above mentioned, and such tracheids may be laid down both over the ends of the medullary rays and over the recently formed phloem-like parenchyma.   It is clear, therefore, that the cells of the pericycle, whether external or internal to the fibers, under stimulation of host infection, are so endowed with meristematic powers as to form new tissues, lignified or parenchymatous, irrespective of their ultimate position in relation to the pericycle fibers.
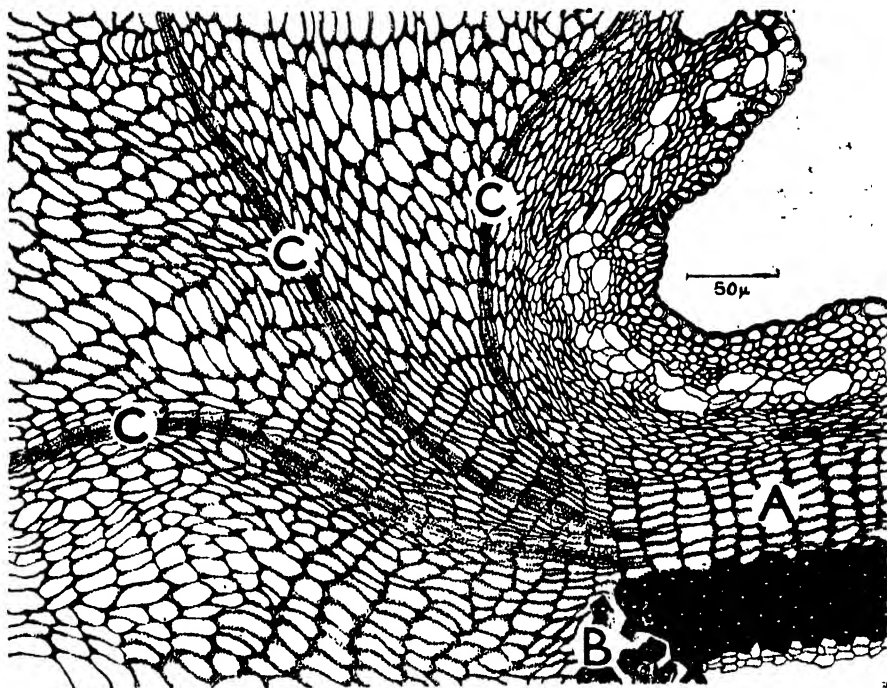
FIG. 4.   Trans. section of portion of stem near junction of gall and stem.   The tissues curving to the right are the stem epidermis, with crushed collenchyma and cortex. (A) the outer pericyclic cambium forming regular rows of parenchyma externally.   (B) some fibers of the pericycle which have become separated probably by pressure of the cambium above; such gaps may become filled with tracheids (See figure 2).   At (C) the secondary parenchyma derived from (A) has fanned out into the gall and, within it, three cambial arcs have become differentiated, forming secondary parenchyma towards both sides.

A striking feature following upon the accumulation of lignified tracheids over the ends of the medullary rays, as above described, is the appearance of one or two deep indentations in the contour of the normal cambium of the woody cylinder (Fig. 1, D), opposite to the rays concerned.

It is evident that considerable radial pressure is brought to bear upon the surface of the woody axis where these rays impinge upon it, the result

being to cause not only a very definite depression in the cambium but also a disruption of the tissues immediately below, in the vascular cylinder. Portions of vascular tissues may thus be displaced so as to become incorporated within the base of the developing gall itself, but the vascularization of the gall tissues is effected independently of the activities of the wood-cambium.   Meanwhile, such an injury to the vascular cylinder appears to stimulate the living cells of the medullary ray close to the seat of injury to divide so as to form a mass of intrusive parenchyma to heal the rift in the
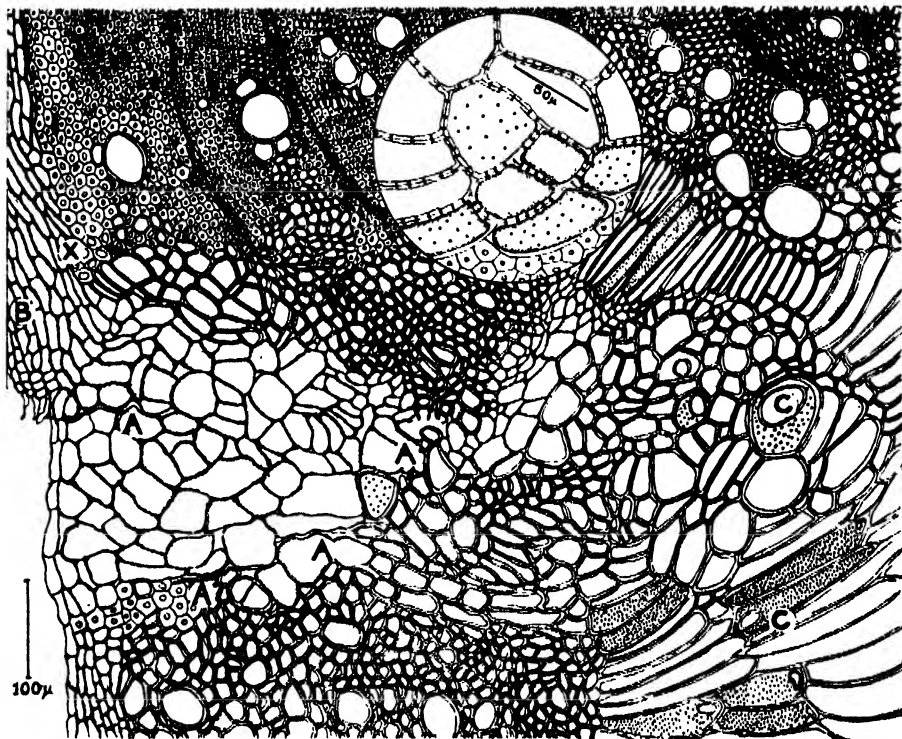
Fig. 5.   Trans. section of portion of stem showing abnormal wood to the right hand, and a wedge of "intrusive parenchyma" to left below (X) (See figure 1, H).   The intrusive tissue has been formed by the medullary ray (B).   At the four places marked (A) are remains of torn lignified elements of the secondary xylem seen above and below. (C) elongated tracheids of the abnormal wood, some converted into short vessels.   Inset, in circle, details of the intrusive tissue, showing empty pitted parenchyma and other cells with thin protoplasmic contents; wood fibers appear below.

axis (Figs. 1, H and 5).   This parenchyma is made up of thick-walled cells most of which are pitted and empty while others without pits are furnished with scanty protoplasmic contents.   In no instance was the intrusive parenchyma seen to have become lignified, and though, perhaps, it may be placed in the category of "abnormal wood," it remained conspicuously clear in all the preparations stained to give lignin reaction.   Moreover, evidences of disruption in the axial cylinder were seen at various places on the boundary

between the intrusive parenchyma and the permanent tissues of the axis, in the form of remnants of lignified wall from the broken tissues (Fig. 5, A).

Meanwhile, in the vicinity of that portion of the wood-cambium indented by external pressure from the thickened, wedge-shaped end of the medullary ray, wide bands of abnormal wood consisting of fibers and tracheids, are, in consequence of the injury to the axis, being swept out into the base of the gall (Fig. 1, F, G). The elements in these lignified bands are disposed tangentially to the surface of the woody axis, as seen in transverse section. It is not easy to trace the formation of these wide sweeps of lignified tissues from axis to gall. They would appear to be formed from cells of the medullary rays bordering the rift in the wood, in the same way as the intrusive parenchyma, but the wood-cambium may also play a part in their formation, should a portion of it become displaced by radial pressure as to sweep into the breach made in the cylinder. As pointed out by Riker (6), it appears that all the living tissues which have not become lignified seem able to respond to the stimulus supplied by the crown-gall organism.

By far the greater bulk of the gall appears to be traceable to the activity of the outer pericyclic cambium. It is true that portions of the host axis, such as displaced vascular bundles and also outgoing leaf traces may become incorporated within the base of the gall (Fig. 1, E, and Fig. 6, H), but all the tissues of the tumor, whether lignified or parenchymatous, are derived from the active cells of the pericycle. Whilst, at first, the cells formed by it, which may be called the "secondary parenchyma," are constrained to accumulate in compact layers outside the pericyclic fibers, later, as soon as the surface of the stem has become broken from internal pressure, these layers of secondary parenchyma, no longer under restraint, divide apace to form the bulky tissue of the gall. But cell division in the extruded mass of parenchyma appears to be localized at various points within it so that numerous cambial arcs arise here and there within the gall (Fig. 6, C, E, F, G, H). While at the junction of gall and stem, a number of cambial arcs fanning out into the tumor may be seen to have a common origin in the outer pericyclic cambium, in the gall itself the meristematic arcs are scattered throughout the parenchyma from which they are differentiated. With the general expansion of the gall, as fresh masses of parenchyma accumulate, fresh cambial arcs again become differentiated from the parenchyma. It follows, therefore, that within the confines of the gall, these constantly recurring arcs of meristematic tissue must contend one against the other to such an extent that while the intervening bands of parenchyma are being converted into lignified tracheids they also suffer great distortion in the making. Where crushed parenchyma has been killed and obliterated, large cavities of diverse shape tend to appear in the expanding gall.

The most striking feature in the mature galls is, of course, the intricate vascularization by lignified tracheids and vessels. These lignified elements, often greatly distorted during formation, present such an intricate pattern amongst the thin-walled parenchyma of the gall as to preclude the attempt

FIG. 6. Section of a portion of a gall showing general structure. (A) inset circle, the bacteria in a pocket at surface of gall, as at (B) and (C). At (C, E, F, G, H) and other places, note portions of meristem or cambial arcs forming secondary parenchyma. Note the variously shaped groups of lignified tracheids, some spherical as at (E), others V- or Y-shaped. (D) details of a spherical group of tracheids formed around a small group of parenchyma. Just below (H) is a leaf-trace pushed out into the gall and subsequently invested by a cambial meristem which has formed a few lignified tracheids towards the leaf-trace together with secondary parenchyma towards the gall. Note the crushing and tearing of gall parenchyma, with cavities, due to the contending activities of the numerous cambial arcs.

to describe any common type of formation. But they are all cells of the
secondary parenchyma converted by pitting and lignification of the walls
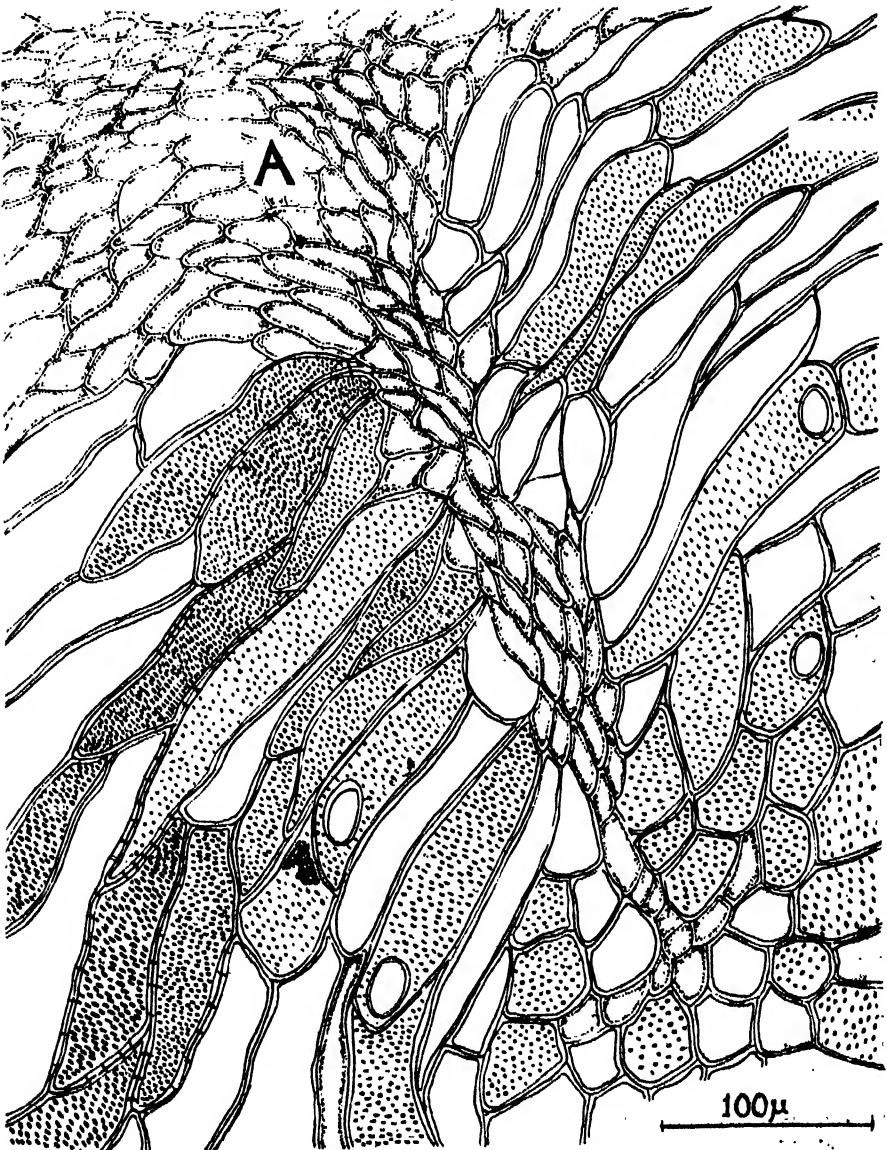
FIG. 7. Portion of gall showing secondary parenchyma forming elongated elements
which become converted into lignified tracheids. At (A) the parenchyma is undifferen-
tiated but sweeps out below in two directions to form the tracheids, leaving medullary
ray-like parenchyma in between.

into vascular elements, the vessels being produced by mere perforation of
the cross wall between the tracheids (Fig. 6, D) ; they do not attain the same
degree of thickness and lignification of the walls as observed in the elements
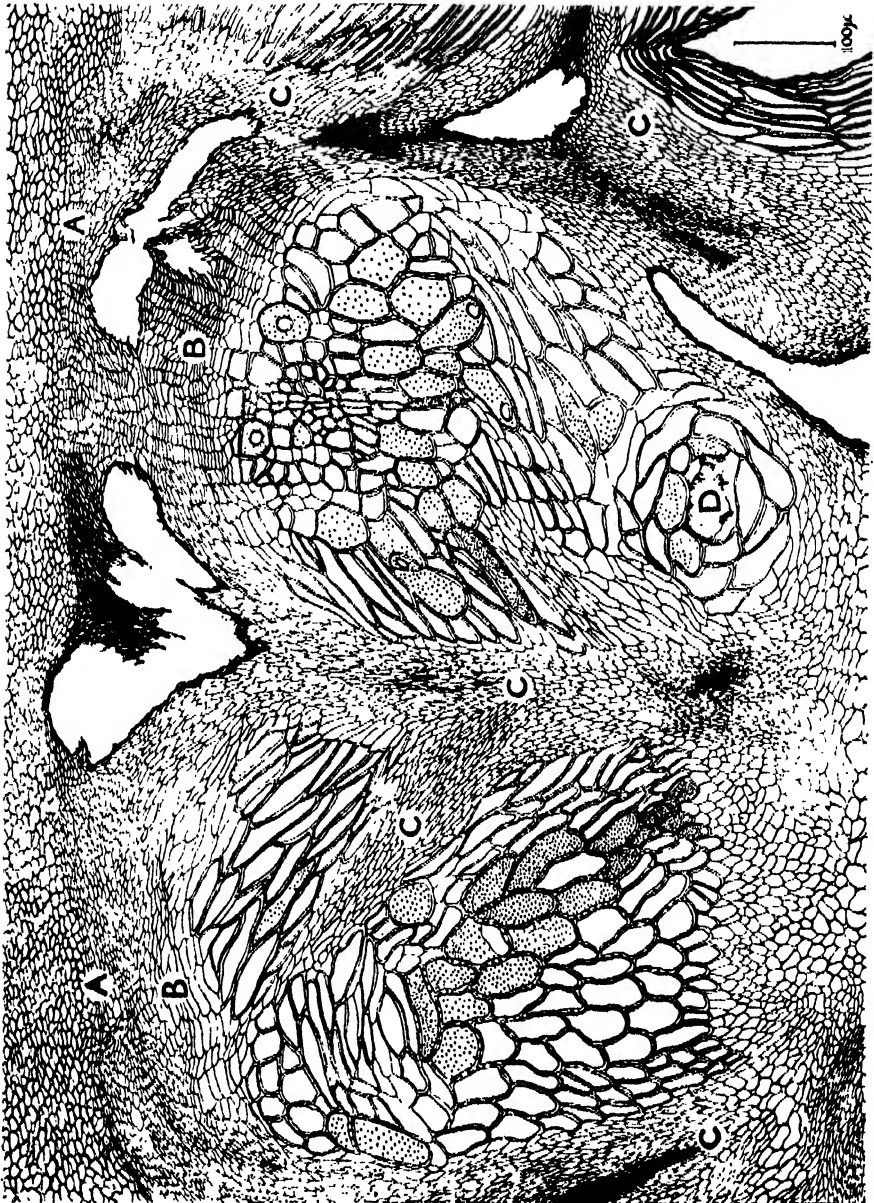
Fɪɢ. 8. Section of a gall, near periphery. The long sweep from (A) to (A) is a single cambial arc which has formed towards the interior a number of ''bundles'' arranged, more or less, in a ring. Below (B) and (B) are two ''bundles'' separated by secondary parenchyma resembling medullary rays, as at the five places marked (C). But the ''bundles'' are merely groups of tracheids, with radial rows of phloem-like cells above, all derived from secondary parenchyma. (D) a small, spherical group of tracheids formed around a pocket of the bacteria.

in the secondary xylem óf the axis.   At various points in the gall large masses of the altered parenchyma become elongated in the same direction, so that spindle-shaped groups of tracheids are formed (Fig. 7) ; at other places such groups in course of formation have evidently become bent or twisted with the result that, round, or Y-, or X-shaped groups, or other fantastic forms of the lignified tissues may be found, as a result, no doubt, of the contending activities of the numerous cambial arcs in the gall. . Again, a common feature, towards the periphery of the gall, is the appearance of apparently.complete vascular bundles, like those of an herbaceous dicotyledon, but without fibers (Fig. 8, B, B).   This similarity is further enhanced when such bundles, arising close together, are separated by radial rows of .parenchyma, like medullary rays (Fig. 8, C).   But similar bundles may frequently be seen in comparative isolation at variable depths from the surface of the gall. Such pseudo-bundles have also been observed by Butler (1), Riker (6), and Smith, a feature described by Smith (8) as ''a stem within a stem.''   These bundles have, however, no connection with the axial cylinder of the host stem ; they are merely aggregations of converted secondary parenchyma in which some of the cells become lignified tracheids, while others, smaller, arranged in more or less radial rows over the tracheidal groups, have all the appearance of a normal phloem.   In the present study these pseudo-bundles, when arising close together below the periphery of the gall, are observed to be in close association above, with a long sweep of cambial meristem in the outlying parenchyma (Fig. 8, A, A).   Extending inwards from this cambial arc, all stages in the transition of the derived secondary parenchyma into lignified tracheids and phloem-like cells may be seen (Fig. 8, B), together with bands of parenchyma passing in between the bundles, in the manner of medullary rays.   The fact that such ''ray'' parenchyma may also become transformed into lignified tracheids is further evidence of a common origin of all parts of the pseudo-bundles from the secondary parenchyma (Fig. 7).

DISCUSSION

Butler (1) and Riker (7) suggest that the peculiar distribution of the lignified elements in the gall, may be due to the way the bacteria (or, perhaps their infiltrating products, or associated growth substances) may collect in various parts of the gall.   If the bacteria are confined to a restricted space, the stimulus of infection, localized around such a focus, would result in the conversion of a small group of parenchyma into tracheids (Fig. 8, D), a feature which has been observed very frequently in the present study. When groups of isolated tracheids occur, it is further suggested that such distribution may have resulted from stimulation from several directions at one time.   Furthermore, when the gall tracheids are assembled with more or less regularity, in the manner of vascular bundles, it is conceivable that the stimulus from a single locus is maintained in one direction, resulting in a definite orientation of the tissues; in the absence of sustained stimulation from a particular source, but from a number of places sufficiently separated, a regularity in the lignified pattern would hardly be expected.

Adopting the views put forward by Butler and Riker (1, 7), the present writer suggests that the cambial arcs within the gall, as revealed in the present study, are actually the foci from which the vascularization of the gall is directed.

SUMMARY

The sub-aerial galls caused by *Bacterium tumefaciens* in natural infection on the stems of the Himalaya giant blackberry (*Rubus procerus*) originate from the outer layers of the pericycle.

Increments of lignified tracheids to the terminations of the medullary rays in the vicinity of the gall are instrumental in causing a disruption of the host tissues.

The distribution of the tracheids within the gall appears to be due to the activity of numerous meristematic zones or cambial arcs in the gall parenchyma.

DEPARTMENT OF BOTANY,
　UNIVERSITY OF GLASGOW,
　　SCOTLAND.

LITERATURE CITED

1. BUTLER, E. J. Some aspects of the morbid anatomy of plants. Ann. App. Biol. 17: 175–212. 1930.
2. HILL, J. BEN. The migration of *Bacterium tumefaciens* in the tissue of tomato plants. Phytopath. 18: 553–564. 1928.
3. MAGROU, J. Études sur les galles produites par le *Bacterium tumefaciens*. Ann. Sci. Nat. Bot. Ser. 10. 10: 545–582. 1928.
4. ROBINSON, WILFRED, and H. WALKDEN. A critical study of crown gall. Ann. Bot. 37: 299–324. 1923.
5. RIKER, A. J. Some relations of the crowngall organism to its host tissue. Jour. Agr. Res. [U.S.] 25: 119–132. 1923.
6. ————. Some morphological responses of the host tissue to the crowngall organism. Jour. Agr. Res. [U.S.] 26: 425–435. 1923.
7. ————. Cytological studies of crowngall tissue. Amer. Jour. Bot. 14: 25–37. 1927.
8. SMITH, ERWIN F. Studies on the crown gall of plants. Its relation to human cancer. Jour. Cancer Res. 1: 231–309. 1916.
9. ————. Appositional growth in crown-gall tumors and in cancers. Jour. Cancer Res. 7: 1–105. 1922.
10. ————, NELLIE A. BROWN, and LUCIA McCULLOCH. The structure and development of crown gall: a plant cancer. U. S. Dept. Agr., Bur. Pl. Indus., Bull. 255. 1912.

THE CONTROL OF LATE BLIGHT IN TOMATO SEED–BEDS UNDER EPIPHYTOTIC CONDITIONS

A. L. HARRISON

The unusually severe epiphytotics of late blight caused by *Phytophthora infestans* (Mont.) de Bary that occurred during the spring of 1946 have already been reported.[1] The disease was first observed late in November, but it was not until the middle of January that it became widespread and extremely destructive in many areas of the State. Two periods of weather during the middle and latter part of January were particularly favorable for its spread and development.[2]

Late blight has been reported only infrequently as occurring naturally on tomato seedlings. It has never been reported on tomato seedlings from the West Coast of Florida so far as the writer is aware. Consequently when the disease made its sudden and destructive appearance in tomato seed-beds in Manatee and Hillsborough counties in January, no one was prepared for the onslaught. During this period all control measures appeared to fail. This apparent failure resulted primarily from the lack of an adequate spray program, failure to get thorough coverage, and failure to start spraying in time. A few growers who consistently followed recommendations secured good plants. Growers who jumped from one spray to another, who did not get coverage, or who did not apply sprays frequently enough lost most of their early plants. Probably 95 per cent of the early seed-bed plants were lost because of late blight during the winter of 1945–46 in this section.

This outbreak of late blight afforded an excellent opportunity to see what could be done for the control of this disease in tomato seed-beds. Consequently, several experiments were conducted using different spray materials.

MATERIALS AND METHODS

When late blight was first found late in the season on the mature fall tomato crop, the Dithane (disodium ethylene bisdithiocarbamate)-zinc sulfate-lime spray which had been so successful in controlling the disease on potatoes in South Florida[3] was urgently suggested to growers for its control on tomatoes despite a lack of specific information with this crop. Consequently, this was one of the sprays used in the tomato seed-bed tests. In all cases in this paper where Dithane is mentioned it was used at the following concentration: Dithane D14, 2 quarts; zinc sulfate, 1 lb.; lime, ½ lb.; and water, 100 gal., unless otherwise stated.

[1] Harrison, A. L. Potato late blight on tomatoes on the west coast of Florida. U. S. Dept. Agr., Pl. Dis. Reptr. **30**: 49–50. 1946.

[2] Harrison, A. L. The relation of weather to epiphytotics of late blight on tomatoes. Phytopath. **37**: 533–538. 1947.

[3] Ruehle, G. D. A new organic fungicide for the control of potato late blight in Florida. Fla. Agr. Expt. Sta. Press Bul. 598. 1944.

In addition, various other organics were used in one or more tests.   They are as follows: DuPont IN 5446 and IN 7331, Rohm and Haas He 178,[4] zinc chromate spray (C.P.I. 169A), Zerlate (zinc dimethyl dithiocarbamate), Phygon (2,3 dichloro 1,4 naphthoquinone), Puratized N5E (phenylmercuri triethanol ammonium lactate), and copper 8-quinolinolate.   Several standard fungicides were also used in the tests for comparison.

The sprays were applied with a Champion knapsack sprayer, care being taken to get thorough coverage on both leaf surfaces and on the stems of the seedlings.   Sprays were started soon after the seedlings emerged.   This was a necessity during the epiphytotic, since seedlings were repeatedly observed to succumb to late blight before the first true leaves had been formed. Moveable screens were used so that there was no spray drift from plot to plot.   The sprays in all tests were continued until the seedlings were ready for field setting.

The individual spray plot varied from 6 to 8 rows, depending on the test, each approximately 2.5 ft. long.   The rows were planted crosswise in outdoor ground beds.   In the first two tests, each spray plot was subdivided so that one half of the plot was sprayed once a week and the other half twice a week.   Each plot contained several hundred plants at the start of the tests.

All treatments were randomized and replicated so that the data could be analyzed according to standard statistical methods.   Efficiency ratings were taken from the different spray treatments at different times during the course of the tests by classifying samples of 25 plants from each plot or subplot (depending on the experiment) into 4 classes, with different arbitrary values given to each class.   Each of the 25 plants was separately classified, and the sums of the separate values totaled to give a quantitative figure for use in the statistical analysis.   A dead plant was rated 0, a severely diseased plant 1, a slightly diseased plant 2, and a plant without blight symptoms was rated 4.   A rating of 100 for a plot would indicate apparently perfect disease control on all 25 plants.   The 25-plant sample was taken from the same relative position from each plot for each date of sampling.   This method of taking data was relatively rapid and proved much more reliable than arbitrarily giving each plot an efficiency rating.

Experiment 1.—The first test was conducted at Ellenton, Florida, on a seed-bed planted on January 25 adjacent to tomato seed-beds that had just been abandoned because of late blight.   Eight applications were made on the subplot receiving sprays twice a week and 4 on the subplots receiving sprays only once a week.   The first spray was applied on February 6, and the last on March 2.   Weather was not particularly favorable for late blight until February 27, although centers of infection were beginning to show in the unsprayed plots on February 23.   The weather from February 27 through March 2 was extremely favorable for the spread and development of late blight and it spread very rapidly through the check plots and over

[4] IN 5446 and He 178 are different formulations of zinc ethylene bisdithiocarbamate. Samples of He 178 were used from different batches and are distinguished as He 178B and He 178E.   IN 7331 is a formulation of manganese ethylene bisdithiocarbamate.

some of the spray plots. Efficiency ratings for the various sprays were made on March 7. The plants at that time were about 6 to 8 inches high.

The data presented in table 1 and figure 1 demonstrate that late blight can be held in check in the seed-bed by Dithane and IN 5446, and that applications should be made twice a week during periods when late blight is extremely active. The degree of control obtained with Copper-A Compound was not considered satisfactory even when the copper was applied twice a week.

The investigation was carried one step further because growers had been experiencing extreme difficulty in getting tomato plants to live after being transplanted in the field even though the plants had been pulled from beds that were relatively free of the disease. For this study, samples of 60 appar-

TABLE 1.—*Effect of some fungicides on the control of late blight in tomato seed-beds, Experiment 1*

Treatment	Spray efficiency ratings		
	Weekly[a]	Semiweekly[a]	Spray totals[b]
Dithane, zinc sulfate, and lime:			
2 qt.-1 lb.-0.5 lb.-100 gal.	205	260	465
IN 5446: 1.25 lb.-100 gal.	179	256	435
Copper-A: 4 lb.-100 gal.	124	182	306
C.P.I. 169A: 4 lb.-100 gal.	74	148	222
Zerlate: 2 lb.-100 gal.	60	75	135
Check	0	0	0
Frequency Totals[b]	642	921	

[a] Maximum rating possible for each treatment 300 (3 replications).
[b] Least differences required for significance at 19 : 1—Spray totals, 84; Frequency totals, 125.

ently healthy plants were pulled from the plots sprayed with Dithane once and twice a week and from the plots sprayed twice a week with IN 5446. The samples were pulled on March 7 (the same day the spray efficiency ratings were made) and set in steam-sterilized soil. Samples were not obtained from any of the other plots because of the difficulty in getting plants even apparently free of late blight. Only 4 plants survived among the 60 set from the plot sprayed once a week with Dithane. Forty-two survived among those set from the plot sprayed twice a week with Dithane, and 48 survived from the plot sprayed twice a week with IN 5446. Thus when late blight is active there is a strong possibility of losing the plants in the field unless the plants have been frequently and thoroughly sprayed with the proper materials.

Experiment 2.—The second test was conducted at the Vegetable Crops Laboratory, Bradenton, Florida, in beds planted February 1. Ten applications were made on the subplots receiving sprays twice a week and 5 on the subplots receiving sprays only once a week. The first spray was made on February 13 and the last on March 16. Three treatments were discontinued after the application of March 6 because of their failure to hold late
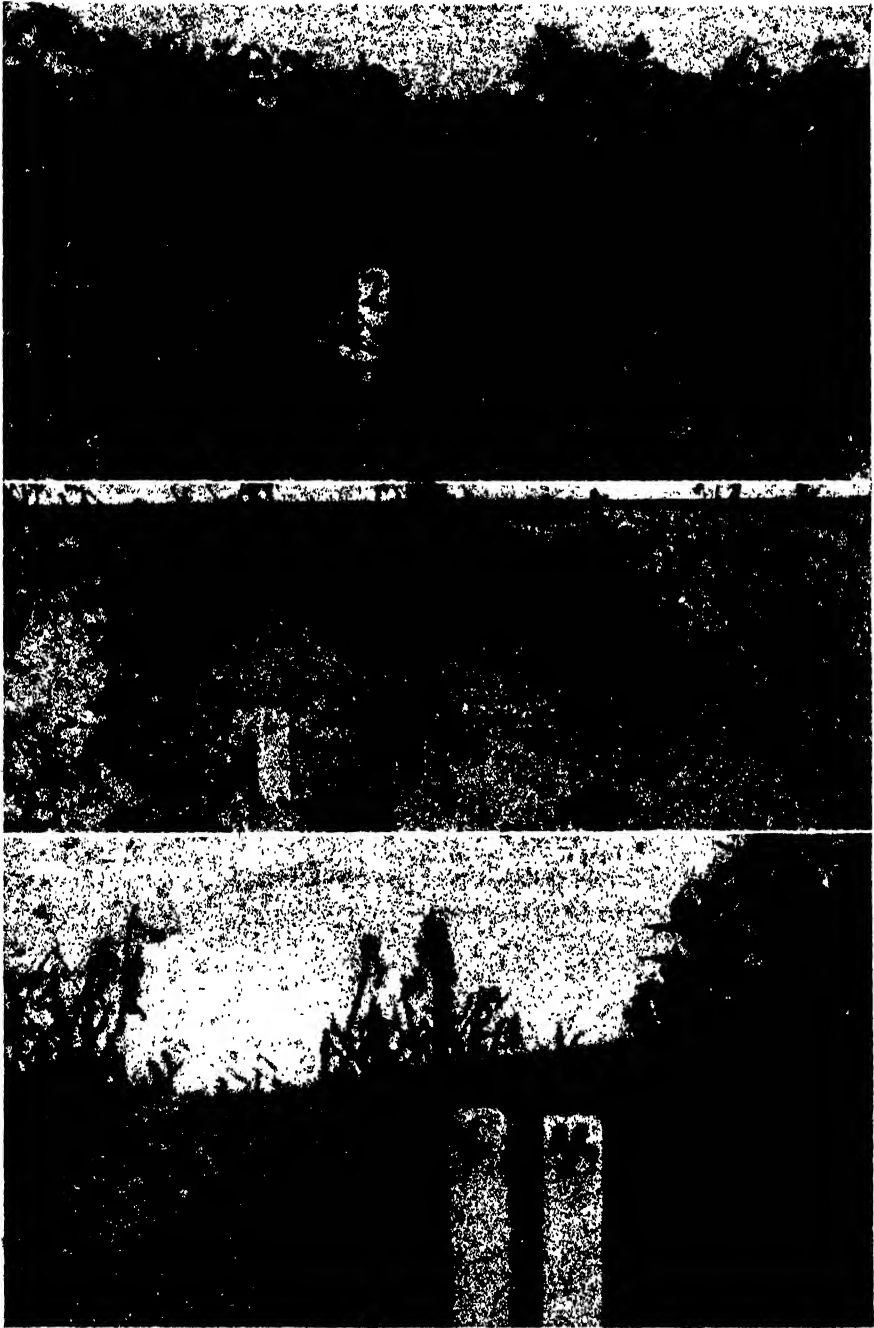
FIG. 1. Effect of some sprays on the control of late blight in tomato seed-beds (Experiment 1). A. Typical plot sprayed twice a week with Dithane, zinc sulfate, and lime. B. Unsprayed plot. C. Portion of unsprayed plot on the left and on the right a portion of plot sprayed once a week with IN 5446. Photographed March 5 by D. G. A. Kelbert.

blight in check. When the weather turned favorable for late blight on February 27, tomato leaves with active blight lesions were scattered uniformly around and through all plots in the experiment. The weather continued to be favorable for late blight until March 2 and it spread very rapidly through the unsprayed plots and some of the sprayed plots. Harrison[5] gives a detailed description of the weather during this period. Late blight was first observed on March 1 in these seed-beds. Spray efficiency ratings were taken on March 5, 15, 21, and 30. The data are presented in table 2 and verify the results from the first experiment, namely, that Dithane was the best material used for the control of late blight. The substitution of ammonia for lime in the spray mix did not impair the fungicidal efficiency of Dithane. The material IN 5446 applied twice a week was as good as Dithane once a week, but not so good as Dithane twice a week. He 178 was not so good as IN 5446. Copper-A compound again failed to satisfactorily hold late blight in check.

As in the first experiment, plants from some of the plots were pulled and set in steamed soil for a check on plant survival. In addition, the plants were dipped in various fungicides to see if this would stop late blight from developing on the transplants. Plants for these studies were pulled from the standard Dithane, IN 5446, and Copper-A plots. The plants were sorted, and all plants with observable leaf or stem lesions of late blight were removed. Two separate tests were conducted. In the first test, the plants were pulled on March 21, sorted and dipped, both roots and tops, in the various fungicides, wrapped in kraft paper and set aside 16–17 hours before setting in the steamed soil. The results (Table 3) were taken on April 5. The second test was a duplication of the first except that the plants were pulled early in the morning of March 25, sorted, dipped, and held for 24 hours before setting in the steamed soil. The results of this test are also presented in table 3. The loss of plants in both tests was due primarily to late blight.

The seed-bed spray has a marked influence on controlling late blight in tomato transplants. More of the Dithane-sprayed plants survived transplanting than did the plants sprayed with Copper-A. The differences were significant in both tests. The differences between the plants sprayed with Dithane and those sprayed with IN 5446 were significant in favor of the Dithane in one test, but not in the other. Plants from the Dithane-sprayed plots were heavier in both tests. There was no significant difference in weight between the plants sprayed with Copper-A and plants sprayed with IN 5446.

IN 7331, Phygon, and Dithane all significantly reduced the loss from late blight in the transplanting operation. IN 5446 caused a significant reduction in one test but not in the other. Plants dipped in Copper-A were not significantly better than the check on the basis of plant survival.

The plants dipped in Dithane were significantly smaller than the plants

TABLE 2.—*Effect of some fungicides on the control of late blight in tomato seed-beds, Experiment 2*

| Treatment | Spray efficiency rating[a] | | | | | | | | | | | |
| | March 5 | | | March 15 | | | March 21 | | | March 30 | | |
	Weekly	Semi-weekly	Spray total	Weekly	Semi-weekly	Spray total	Weekly	Semi-weekly	Spray total	Weekly	Semi-weekly	Spray total
Dithane Ammonia[b]	400	400	800	348	392	740	328	394	722	352	398	750
Dithane (Standard)	380	400	780	340	379	719	364	392	756	315	383	698
IN 5446: 1.25 lb.–100 gal.	400	400	800	288	367	655	221	315	536	200	349	549
He-178B: 1.25 lb.–100 gal.	340	380	720	226	289	515	163	253	416	180	265	445
Copper-A: 4 lb.–100 gal.	360	360	720	300	348	648	190	259	449	167	233	400
C.P.I. 169A: 4 lb.–100 gal.	200	260	460	78	163	241	......	......	......	......	......	......
Zerlate: 2 lb.–100 gal.	160	240	400	49	113	162	......	......	......	......	......	......
New Improved Ceresan: 1 lb.–250 gal.	140	200	340	52	98	150	0	0	0	0	0	0
Check	40	40	80	0	0	0	0	0	0	0	0	0
Frequency totals	2420	2680	......	1681	2149	......	1266	1613	......	1214	1628	......
Least diff. for sig. at 19:1 between totals	89	124	......	188	132	......	126	131	......	150	103	......

[a] Maximum rating possible for each treatment 400 (4 replications).
[b] Commercial ammonia (2 qts. in 100 gal. spray) replaced the lime in this Dithane spray.

from most of the other treatments. Phygon as a dip also caused some injury. IN 7331, IN 5446, and Copper-A as dips were the best from the standpoint of plant growth following the transplanting operation.

TABLE 3.—*Effect of seed-bed sprays and plant dips on survival and weight of tomato transplants, Experiment 2*

Plant dip	Seed-bed spray	Number of plants surviving in test[a]				Relative weights in grams of plants in test[b]			
		1		2		1		2	
		Interaction Dip × Spray	Dip	Interaction Dip × Spray	Dip	Interaction Dip × Spray	Dip	Interaction Dip × Spray	Dip
IN 7331:	Copper-A	39		27		15.9		13.7	
1.5 lb.–100 gal.	Dithane	43		29		18.1		17.1	
	IN 5446	39		26		13.8		13.5	
			121		82		47.8		44.3
Phygon:	Copper-A	36		27		14.2		9.8	
1 lb.–100 gal.	Dithane	40		28		16.4		14.2	
	IN 5446	36		28		13.7		12.2	
			112		83		44.3		36.2
Dithane Standard	Copper-A	26		22		10.0		12.6	
	Dithane	45		28		15.6		11.3	
	IN 5446	38		27		11.1		7.4	
			109		77		36.7		31.3
IN 5446:	Copper-A	30		22		17.7		10.5	
1.75 lb.–100 gal.	Dithane	42		30		17.7		17.3	
	IN 5446	27		28		20.4		15.5	
			99		80		55.8		43.3
Copper-A:	Copper-A	26		18		17.4		9.0	
4 lb.–100 gal.	Dithane	43		30		15.2		18.5	
	IN 5446	27		25		16.9		10.4	
			96		73		49.5		37.9
Water	Copper-A	26		17		14.1		16.4	
	Dithane	38		22		23.2		11.0	
	IN 5446	27		22		19.8		11.6	
			91		61		57.1		39.0
Least diff. between totals for sig. at 19:1		N.S.	18.4	N.S.	13.8	N.S.	8.3	4.0	7.0
Seed-bed spray totals combined		Test 1		Test 2		Test 1		Test 2	
Copper-A		183		133		89.3		72.0	
Dithane		251		167		106.2		89.4	
IN 5446		194		156		95.7		70.6	
Least difference between totals for significance at 19:1		26.0		19.5		11.7		9.9	

[a] In the first test there were three replications of 15 plants; in the second test, three replications of 10 plants each.
[b] The interaction figures are totals of three average weights of plants in the respective treatments.

Experiment 3.—A third experiment for the control of late blight in seedbeds was conducted on beds planted March 15 beside the beds used in the second spray test. Five fungicide applications were made from March 25

to April 11. The frequency of application was determined by the rate of growth of the tomato seedlings and the weather. Late blight was relatively inactive in the field for the duration of the test. However, by sprinkling the beds late in the evening with a spore suspension of *Phytophthora infestans,* some late blight developed in all plots. This was done twice during the duration of the test. The suspension was obtained by washing the spores from blighted tomato leaves that had been kept in moist chambers for 2–3 days. It was applied as uniformly as possible to all plots by means of a sprinkling can. Spray efficiency ratings were taken on April 13 and 23, and are presented in table 4. The data again show that Dithane and the dithiocarbamate sprays IN 5446 and He 178 are definitely superior to the copper sprays for controlling late blight on tomato seedlings in Florida. Phygon also was a promising fungicide for late-blight control in this test.

TABLE 4.—*Effect of some fungicides on the control of late blight in tomato seed-beds, Experiment 3*

Treatment	Spray efficiency rating[a]	
	Apr. 13	Apr. 23
Phygon: 0.5 lb.–100 gal.[b]	300	274
IN 5446: 1.75 lb.–100 gal.	300	272
Dithane, zinc sulfate, and ammonia 2 qts.–1 lb.–1 qt.–100 gal.	300	268
He 178E: 1.75 lb.–100 gal.	300	260
Dithane, zinc sulfate, lime 2 qts.–1 lb.–0.5 lb.–100 gal.	300	254
Stand. Dithane and Copper-A alternating	300	238
Dithane, zinc sulfate, lime, and ammonia 2 qts.–1 lb.–0.5 lb.–1 qt.–100 gal.	300	236
IN 7331: 1.5 lb.–100 gal.	296	222
Super Copper:[c]	288	205
Copper-A: 4 lb.–100 gal.	272	201
Copper 8-quinolinolate: 1 lb.–100 gal.	273	176
Puratized N5E[d]	201	130
Check	137	62
Least differences between totals for significance at 19: 1	36	34

[a] Maximum rating possible for each treatment 300 (3 replications).
[b] First application made at 1–100.
[c] A liquid material containing copper sulfate and ammonia. Used at 1 gal.–400 for the first two applications. All others at the rate of 1 gal.–300.
[d] At first used at 1 part to 2000 of a 10 per cent solution but later cut to 1 part to 4000 because of plant injury.

DISCUSSION

The data presented in tables 1, 2, and 4 demonstrate that late blight may be held in check in tomato seed-beds even under conditions extremely favorable for the spread and development of *Phytophthora infestans.* This is illustrated in figure 1. Both experiments 1 and 2 were conducted during one of the periods, February 27 through March 2, when late blight was extremely active. In experiment 2, tomato leaves with active late blight lesions were scattered over the plots during this period in order to give the spray materials the severest type of test. Even so, several sprays were outstanding in their performance in holding late blight in check.

The outstanding spray material in the first two tests was the mixture of Dithane, zinc sulfate, and lime. The materials IN 5446 and He 178 which are different formulations of zinc ethylene bisdithiocarbamate gave good control of late blight in the first two tests in spite of the fact that they were not used at strengths equivalent to the Dithane spray. In the third experiment where the dithiocarbamate content of Dithane, IN 5446, and He 178 were at approximately equal concentrations, the spray efficiency ratings are approximately equal. Altering the Dithane spray formula with commercial liquid ammonia did not materially effect the results, except that the addition of both lime and ammonia to the spray mix was very injurious to the tomato plants.

Phygon was used only in the third test where it showed considerable promise for the control of late blight. The first application had been made at 1–100 but was cut to 0.5–100 because of some flecking damage or burning on the leaves. There was no injury evident at the reduced concentration.

All copper sprays controlled late blight fairly well while the seedlings were very small and when it was easy to obtain good coverage, but just as soon as the plants became crowded, late blight started to spread rapidly over the copper sprayed plots. In some tests, on the survival of plants from the various sprayed plots during the transplanting operation, it was extremely difficult to find plants without lesions on the copper plots, while it was comparatively easy to find blight-free plants on the plots sprayed with Dithane and IN 5446.

The only other material used that gave any promise as a fungicide for the control of late blight was IN 7331. This was used in only one test and was about equivalent to the copper sprays.

The materials Zerlate, zinc chromate (C.P.I. 169A), New Improved Ceresan, and Puratized N5E gave little or no control of late blight.

In practically all cases sprays applied twice a week were more efficient in controlling late blight than were sprays applied once a week.

Although Dithane consistently controlled late blight it has one distinct disadvantage as far as tomato seedlings are concerned. It injures young tomato plants. Dithane-sprayed plants were short and stocky with leaves that resembled a mild case of shoe-string mosaic (Fig. 1). In severe cases the terminal bud was destroyed. The severity of injury was related to temperature, frequency of application, and quantity of material applied. High temperature and heavy or frequent application of sprays increased Dithane injury. Dithane-sprayed seedlings were delayed a few days but fewer plants were lost from late blight during the transplanting operation than when the plants were sprayed with Copper-A. No injury has been observed on large tomato plants when Dithane has been applied at weekly intervals at the strengths used in these tests.

The fungicides IN 5446 and He 178 did not injure young tomato seedlings even when applied as often as twice a week. These materials have a wide margin of plant safety and show some promise as plant dips for the control of late blight on tomato transplants.

SUMMARY

Dithane plus zinc sulfate and lime, IN 5446, and He 178 consistently gave good disease control during an epiphytotic of late blight caused by *Phytophthora infestans* on tomato seedlings in outdoor seed-beds. Phygon in the one test in which it was used also gave good control of late blight. None of the copper sprays used was equal to the materials mentioned. All copper sprays held late blight in check for a while but as soon as the tomato seedlings became crowded in the seed-bed, late blight spread rapidly over the copper plots, if the weather was favorable for the disease.

Dithane caused some injury on tomato seedlings. No injury has been observed from IN 5446 or He 178, even when applied twice a week on young seedlings soon after emergence.

IN 7331, Dithane plus zinc sulfate and lime, Phygon, and IN 5446 as dips all helped to reduce the loss of tomato plants from late blight during the transplanting operation, especially when the plants had previously been sprayed in the seed-bed with Dithane plus zinc sulfate and lime or IN 5446.

VEGETABLE CROPS LABORATORY,
    FLORIDA AGRICULTURAL EXPERIMENT STATION,
        BRADENTON, FLORIDA.

IMPROVED METHODS FOR THE CULTIVATION AND STORAGE OF PHYTOPHTHORA INFESTANS[1]

S . F . S N I E S Z K O ,[2] J . B . C A R P E N T E R ,[3]
E . P . L O W E ,[3] A N D J . G . J A K O B[4]

INTRODUCTION

Successful control of late blight of potatoes and tomatoes depends chiefly on the proper application of preventive measures, the most important of which are: (1) sanitary precautions eliminating the sources of infections and preventing the spread of the disease; (2) chemical means of plant protection and destruction of the pathogen; (3) breeding of resistant and immune varieties of crops. In the evaluation of control measures and in epiphytological studies, it is necessary to have a steady and dependable supply of the pathogen in a viable and virulent form. This paper describes the results of studies on the development of methods of production and storage of the sporangia and mycelium of *Phytophthora infestans* (Mont.) De Bary.

The substrate generally employed by the early investigators consisted of whole live potato tubers and leaves (6), slices of raw potato tubers, or muskmelon or pumpkin fruit (19). Clinton (1, 2, 3) tried over 70 different media, consisting mostly of various plant materials. The fungus grew best on extracts or suspensions of ground lima beans, with the addition of extracts from ground cereal seeds. Soil and manure supported only scanty growth. Heat-sterilized potato tuber tissue was unsatisfactory. De Bruyn (8) also found that many plant materials served as excellent media. Old dried stems of plants were better than fresh ones. She reported (7) that the fungus would grow fairly well in bog soil or clay, and poorly in leaf mould and sand. Potato tuber tissue in the form of aseptically removed plugs was recommended by Jones *et al.* (10). For many years, this medium was considered the best for production of sporangia. Liquid media tried by Jones *et al.* (10) and Kossowicz (11 to 18) were more suitable for the production of mycelium than of sporangia.

In the present studies separate types of media were developed for the production of sporangia and mycelium. Solid substrates were better adapted for the development of sporangia, whereas the growth and harvesting of mycelium was accomplished more easily in liquid media. Since the experiments on cultivation were closely interwoven with storage and longevity tests, the results are described in two separate sections. One section

deals with sporangia grown on solid substrates and the other with mycelium grown in liquid media.

<center>PRODUCTION OF SPORANGIA</center>

Phytophthora infestans, like most fungi, produces sporangia abundantly only if sporangiophores can develop in the air above the surface of the medium. Some fungi, such as *Penicillium* (9), produce conidiospores abundantly in submerged culture provided the medium is vigorously aerated. It was expected that best sporangial yields of *Phytophthora* could be obtained on a porous substrate in which the growth-supporting surface exposed to air would be as large as possible. The cultures were incubated at 20° C.

Culture Media

Criteria for evaluation of the media were as follows: (1) abundance, rate, and uniformity of growth; (2) physical consistency; (3) availability of the ingredients; and (4) ease of preparation. A detailed discussion of the numerous materials tested and the results obtained would require too much space, since only a few were satisfactory for the production of sporangia. It is believed, however, that a list of the tested materials may be of value to future investigators.

Materials which supported good growth but which were not used for the production of sporangia because of undesirable physical properties or difficulties in handling and preparation were as follows: (1) whole seeds of oats, barley, rice, corn, soybean, lima bean, cowpea, pea, and peanuts; (2) meals from peas, peanuts, soybeans, lima beans, cowpeas, cottonseed, oats, barley, rye, wheat, navy beans, corn, corn grits, cracked corn, wheat middlings, wheat bran, malt sprouts, rice polish, rice bran, and malted wheat, oats, and barley; and (3) miscellaneous materials, as "Cerophyl," wheat, rice, or oat straw, brewers yeast, tomato waste, oat hulls, dried milk, peat, fine mesh peanut hulls, malt extract, corn steep liquor, molasses, and tomato seed.

The materials not supporting growth were carrot root tissue, Spanish moss (*Tillandsia sp.*), excelsior, alfalfa, rice hulls, soybean straw, sugar-beet tissue, soybean flakes, water-extracted soybean meal, and water-extracted peanut hulls.

Of all the materials tried, most satisfactory and uniform production of sporangia was obtained on media prepared from various combinations of cereal grains and peanut hulls. The media were prepared from grain, 1 part; peanut hulls, 1 or 2 parts; and distilled water, 2 parts per each part of grain and 4 parts per each part of peanut hulls. A few extra ml. of water were added to compensate for losses during preparation. The ingredients were placed in suitable vessels, steamed for 15 minutes, shaken, plugged, and autoclaved at 15 lb. pressure for 20 minutes. To obtain optimum growth, initial and thorough saturation of the medium with water was essential. A medium prepared from wheat, rye, or preferably malted rye, and peanut hulls, referred to as peanut hull-grain medium hereafter, consistently yielded

the highest numbers of sporangia.   The physical consistency of this medium was good, as it offered a large surface for the production of sporangiophores. The yields were calculated on the basis of sporangia per gm. dry wt. of the medium.   The sporangia were suspended in water, strained through cheese cloth, and counted in a Spencer, Improved Neubauer, Bright-Line haemacytometer.

The relative proportions of the peanut hulls to grain influenced the yield of sporangia (Table 1).   The media with a higher wheat content than 1:1 were not satisfactory because of packing and reduction in area for sporulation.   Inasmuch as peanut hull–grain media and liquid media prepared by extracting grain seeds produced excellent growth, several attempts were made to replace the whole grain with grain extracts.   Water extracts of both germinated and nongerminated wheat, rye, and sorghum were added to pea-

TABLE 1.—*Influence of the proportion of the ingredients in peanut hull-wheat medium on the yield of sporangia*

Ingredients and proportion of each[a]	Sporangia per gm. dry wt. of medium[b]	
	Series 1	Series 2
	$\times 10^3$	$\times 10^3$
Peanut hulls	107	150
Peanut hulls and wheat 1:1	610	858
Peanut hulls and wheat 2:1	784	777
Peanut hulls and wheat 4:1	640	680
Peanut hulls and wheat 8:1	313	543

[a] 8 gm. of peanut hulls and respectively, 0, 8, 4, 2, and 1 gm. of wheat.
[b] Numbers represent average yields from quadruplicate samples.

nut hulls.   Fourteen days after inoculation no growth of *Phytophthora infestans* was visible on the extract media, although growth was abundant in the peanut hull–wheat seed control flasks.   There was no explanation found for these peculiar results.

Another excellent and frequently used medium consisted of 4 parts navy bean seed and 1 part peanut hulls.   The beans were soaked in an excess of hot water for 2 hours, peanut hulls were added, and the mixture steamed for 15 to 30 minutes.   The medium was drained, shaken, and a few ml. of water added to compensate for losses during sterilization.   This medium supported abundant growth of the fungus and had desirable physical properties.   Beans alone supported equally abundant growth but packed during processing so that growth was limited to the surface of the medium.   Maximum growth at optimal conditions was obtained after 8 to 10 days.   The development of aerial mycelium was even more abundant than on peanut hull–grain.   However, the number of sporangia was smaller, usually 200,-000 per gm. dry medium.

A suspension of sporangia in distilled water was used most frequently as inoculum.   Fragments of medium containing both mycelium and sporangia, or the mycelial mat removed from liquid media also gave satisfactory results,

if the inoculum did not have to be distributed evenly throughout the substrate.

The suspensions were prepared by washing sporangia from 8- to 20-day-old cultures on peanut hull–grain medium in 250-ml. Erlenmeyer flasks. Sterile distilled water at about 20° C. was used. The sporangia in such suspensions were about 90 per cent viable. An inoculum containing at least 10,000 sporangia per ml. and equivalent in quantity to 5 to 10 per cent of the wet weight of the medium, produced satisfactory growth of the fungus in about 9 days at 20° C. Although 5 to 10 per cent of inoculum was used to insure greater uniformity in distribution of the sporangia, it appeared that even 2 per cent was satisfactory in small vessels.

Factors Influencing Production and Viability of Sporangia

In most of the tests herein described, the fungus was grown on media consisting of peanut hulls and wheat or rye. The quantity and quality of mycelial growth were evaluated visually and the quantity of sporangia by counting. Under optimal conditions the growth of *Phytophthora infestans* was excellent and the highest yield of sporangia was obtained in 8 to 10 days (Fig. 1).

The duration of the sterilization process had a pronounced effect on growth of the fungus and yield of sporangia. If the medium was autoclaved at 15 lb. pressure for 20 to 30 minutes, the yield of sporangia per gm. of dry medium was about 2,000,000. Sterilization for 40 minutes reduced the yield to about 1,670,000 and for 80 minutes to about 600,000.

Regarding the optimum temperature the observations reported by Jones et al. (10), Vowinckel (22) and Crosier (4) were confirmed; the optimal growth was at 19° to 21° C. At temperatures above 25° C. growth was depressed, and at 30° C. growth ceased.

Maintenance of a high moisture content of solid media was essential for good growth. According to Crosier (5), mycelium was not formed if the relative humidity of the air was below 85 per cent, and the production of sporangia was scant even at 97 per cent. Vowinckel (22) reported that the number of sporangia produced on leaves and tubers of potatoes was best at 100 per cent relative humidity, good at 78, but none were produced at 65 per cent.

This organism is a strict aerobe but because of its slow rate of growth, its oxygen requirements seem to be easily met. No depression of growth due to faulty aeration was ever found in ordinary culture vessels plugged with cotton or glass wool and containing media in layers up to 5 cm. deep. The use of forced aeration in deep cultures did, however, increase yields of sporangia.

In such experiments the peanut hull–grain substrate was placed in one-liter dispensing burettes to a depth of about 50 cm. and autoclaved in an excess of water that was drained off before inoculation. One tube was aerated normally through the cotton plug in the neck of the vessel. The

FIG. 1.   Growth of *Phytophthora infestans* on peanut hull–wheat medium after 10 days' incubation.

other received 50 cc. per minute of humidified, sterile air that entered the vessel at the bottom, and escaped through the cotton plug.   The tubes were kept almost vertical.   The cultures were sampled when 9 days old by removing 100-gm. lots, serially from top to bottom.   Growth in each tube was best near the air inlet.   The relative numbers of sporangia in comparable samples are given in table 2.

TABLE 2.—*The influence of forced aeration on the production of sporangia in deep solid media*

Treatment	Sporangia per ml. of suspension from samples numbered serially from top to bottom of tubes					6
	$\times 10^3$	$\times 10^3$	$\times 10^3$	$\times 10^3$	$\times 10^3$	$\times 10^3$
Normal aeration	5	6	6	4	2	3
Forced aeration	22	46	26	30	32	32
Ratio						
Normal: Forced[a]	1: 4.4	1: 7.5	1: 4.3	1: 7.5	1: 16.0	1: 11.0

[a] Average ratio was about 1: 7.

A simple aeration method based on the density of carbon dioxide gave excellent results. Media consisting of navy beans and peanut hulls were inoculated, inverted, and incubated in this position (Fig. 2).

Phytophthora infestans grew well from pH 5.0 to pH 7.0, preferring the less acid portion of this range. Jones *et al.* (10) found that in too acid or too alkaline media, production of sporangia was depressed faster than was the vegetative growth. The peanut hull–grain media were in the range of pH 5.5 to 6.0 without adjustment. The rate of production of sporangia was based on the yield of sporangia from cultures sampled at intervals after in-
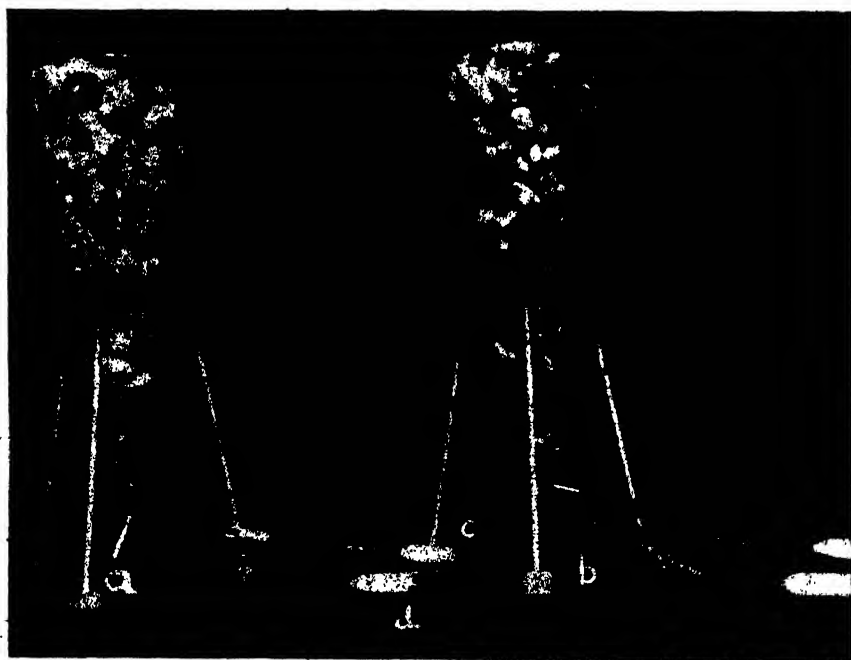
FIG. 2. The growth of *Phytophthora infestans* on peanut hull–grain medium aerated by the gravity-flow system. The air outlet of vessel at left was open continuously; that of vessel at right was closed on the fourth day. Explanation: a, Inoculation port, that contained a cotton-plugged impinger. b, Moisture trap. c, Air inlet-tube extending to the base of the flask. d, Air outlet-tube extending only into the neck of the flask, and protected from air stoppage by the medium by means of a large wire-screen guard.

oculation. In table 3 the yields for 3 experiments are given. The yields for experiments 1 and 2 were low, although the rate of growth was typical. The decrease in number of sporangia through the third day was due probably to the germination and growth of most of the sporangia in the inoculum. Germination tests in experiment 2 indicated this to be true. Most of the sporangia recovered at the 1-, 2-, and 3-day intervals failed to germinate but germination rose to 82 per cent on the fourth day and continued between 80 and 90 per cent throughout the remainder of the experiment. Under optimum conditions the production of sporangia began within 4 days after inoculation. The number of sporangia increased rapidly until about

the ninth day. The apparent decrease in yield of sporangia thereafter was assumed to be due to poor recovery, and mechanical destruction of empty or weakened sporangia.

The ability of sporangia to germinate was the best criterion of their viability. To test germination, about 0.5 ml. of water suspension of sporangia were evenly distributed over the surface of an agar medium in a Petri dish. The best media consisted of 2 per cent extract from peanut hulls or 2 to 3 per cent extract from malted rye with the addition of 2 per cent of agar. In direct germination, two or more germ tubes per sporangium grew to a length of 1 to 2 mm. in 24 hours at about 20° C. Sporangia secured from cultures 7 to 10 days old germinated uniformly at about 90 per cent. The percentage germination was independent of the total number of sporangia produced. Crosier (5) reported that dextrin, Fullers earth, fine quartz, and gelatin increased the germination rate of sporangia. According

TABLE 8.—*The rate of production of sporangia on peanut hull–wheat medium*

| Time | Sporangia per gm. of dry wt. of medium | | |
	Expt. 1	Expt. 2	Expt. 3
Days	$\times 10^4$	$\times 10^4$	$\times 10^4$
1	....	1.3	....
2	1.2	0.8	....
3	0.9	....	1.3
4	9.3	....	4.2
5	36.4	....	27.7
6	66.7	....	74.3
7	68.8	....	129.4
8	62.4	....	207.9
10	....		190.7
12	....	21.7	172.6
14	....	20.0	....

to Uppal (21) some petroleum products stimulated germination. On the media herein reported the germination was uniformly so high that none of the various substances tried had any effect.

The life span of the sporangia of *Phytophthora infestans* is rather short. Therefore, it was considered essential to develop methods that would enhance the survival of sporangia in storage. Sporangia were either stored *in situ* in the undisturbed culture, or were harvested and stored in various substrates. Several hundred flasks containing peanut hull–wheat medium were inoculated and incubated as usual. After 8 to 10 days, some flasks were sampled to determine the number and viability of the sporangia. The number of sporangia per gm. dry weight of the medium varied from 1 to 2 millions. The percentage viability was 80.5 to 93.5 with an average of about 90. Ten days after the inoculation of the media the cultures were divided into 2 series. One was left in the incubator at 20° C. and the other was incubated at 6° to 12° C. Every few days several flasks of each series were sampled to determine yields and viability of the sporangia. Mean figures

representing germination of sporangia in relation to the period and the temperature of the storage are given in table 4.

The viability of the sporangia decreased at first more rapidly in the cultures which were transferred to the lower temperature than in those which were maintained at 20° C. However, the percentage of viable sporangia after 60 days' storage was about the same in both cases.

The viability in storage of sporangia removed from the original culture medium was also investigated. Sporangia were harvested from 8- to 10-day-old cultures, centrifuged, and suspended in sterile distilled water, with and without various supplements. Storage was possible only at temperatures low enough to prevent germination. Of the supplements, only dextrose prolonged the viability of the sporangia.

After 5 days' storage in distilled water, nearly half the sporangia were no longer viable (Table 5). Dextrose in proper concentration approxi-

TABLE 4.—*The longevity of sporangia stored in situ*

Storage period[a]	Germination of sporangia stored at	
	20° C.	6° to 12° C.
Days	*Pct.*[b]	*Pct.*[b]
10	90	90
15	82	52
20	77	41
30	57	38
40	34	28
50	17	22
60	10	15

[a] The storage periods include the 10-day incubation period at 20° C. for both temperature series.
[b] The figure of 90 per cent represents the germination rate at the end of the incubation period.

mately doubled the length of time that the sporangia remained viable. At temperatures below 0° C. the sporangia died, and at temperatures higher than 2° C. they germinated. At 8° to 10° C. most of the sporangia germinated within 4 days.

Drying of sporangia was not tried, because the evidence furnished by other investigators proved conclusively that sporangia die rapidly if the relative humidity falls even a few per cent below the level of saturation. Freezing, lyophilizing, storage in an atmosphere of carbon dioxide or in vacuum, immersion in water of undisturbed cultures, storage in peanut-hull slurry, all gave unsatisfactory results.

PRODUCTION AND STORAGE OF MYCELIUM

De Bary (6) was the first to report that the so-called "perennial mycelium" of *Phytophthora infestans* in potato tubers was most probably instrumental in perpetuating the fungus from one season to another. He stressed the fact that viable mycelium survived for a long time in infested

potato tissue. De Bruyn (8) found that the fungus remained viable for 2½ to 4 years in media composed of sterilized manure or sterilized bog soil. There was a likelihood that in some of the media the perfect spores were formed (8).

Culture Methods

The growth of *Phytophthora infestans* was studied in various liquid media in both shallow and deep cultures. In liquid media, growth consisted mainly of mycelium. Sporangia were formed in appreciable numbers only if aerial mycelium was produced. Shallow cultures were grown in flasks containing a layer of medium 10 to 15 mm. deep. In most cases the shallow cultures were left undisturbed during the entire period of incubation. Deep cultures were grown in 4-liter Pyrex serum bottles, and the medium was

TABLE 5.—*Germination of sporangia after storage in water and several concentrations of dextrose*

Storage period at 2° C.	Germination of sporangia					
	Distilled water	Dextrose concentration, in per cent				
		5	10	15	20	25
Days	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
0	90a	......	.	......	......	......
5	58	71	78	80	73	37
10	35	59	59	63	57	42
15	20	22	39	45	45	32
20	9	11	23	25	30	31

a This figure represents the percentage germination of sporangia, based on the total number of sporangia present. Using this figure as 100 per cent, all other percentages were calculated therefrom.

aerated by means of sterile compressed air bubbled through the cultures. The media were usually inoculated with a suspension of sporangia in sterile water. Inoculum was secured from cultures grown on peanut hull–grain media and added to the fresh media at the rate of 1 to 2 per cent by volume. Cultures were incubated at 20° C. Extracts from peanut hulls, soybean meal, germinated and nongerminated cereal grains were tried alone, in combinations, and in various concentrations; media were adjusted to about pH 6.0. Papain-digested extract from soybean meal was also tested. In shallow cultures visible growth appeared usually within 24 hours, and, if the cultures were undisturbed, aerial mycelium appeared within 5 to 8 days. No apparent increase in the volume of growth occurred in cultures more than about 2 weeks old. In cultures more than 3 weeks old, parts of the aerial mycelium began to collapse and sink, and within 4 to 5 weeks, little if any aerial mycelium was left. The yield of mycelium was determined quantitatively by filtration and weighing with appropriate corrections for solids in the medium. The best growth and yields were obtained on the extracts prepared from germinated cereal grains, particularly rye or wheat.

TABLE 6.—The relative value of several liquid media for the development of mycelium of Phytophthora infestans

Media[a]	Grams of soluble solids per liter	Days in incubation period	Yields of mycelium[b]	
			Grams per liter of medium	Grams per kg. of raw materials
1. Peanut hull extract, 10 per cent	4.88	13	0.47	4.7
2. Peanut hull extract, 10 per cent + 0.5 per cent dextrose	9.88	13	1.47	14.0
3. Peanut hull extract, 5 per cent	2.44	13	0.20	4.0
4. Peanut hull extract, 5 per cent + 0.5 per cent dextrose	7.44	13	1.30	23.6
5. Peanut hull extract, 2.5 per cent	1.22	13	0.09	3.6
6. Peanut hull extract, 2.5 per cent + 0.5 per cent dextrose	6.22	13	1.16	38.7
7. Extract from nongerminated wheat, 1:5[d]	--	11	1.36	6.8
8. Extract from nongerminated wheat, 1:25	--	11	0.36	14.7
9. Extract from germinated wheat, 1:5 + 0.5 per cent dextrose	--	11	1.66	8.1
10. Extract from germinated wheat, 1:25 + 0.5 per cent dextrose	--	12	0.42	9.3
11. Extract from germinated wheat, 1:5	--	11	0.12	0.6
12. Extract from germinated wheat, 1:25	--	11	0.67	16.7
13. Extract from germinated wheat, 1:5 + 0.5 per cent dextrose	--	12	0.21	1.1
14. Extract from germinated wheat, 1:25 + 0.5 per cent dextrose	--	11	1.61	35.8
15. Corn steep liquor,[e] 1:2 + 0.5 per cent dextrose	9.20	11	0.62	6.2
16. Corn steep liquor, 1:4 + 0.5 per cent dextrose	7.10	11	0.3	6.2
17. Sorghum steep liquor, 1:2 + 0.5 per cent dextrose	8.60	11	1.05	10.5
18. Sorghum steep liquor, 1:4 + 0.5 per cent dextrose	6.80	11	0.54	10.8
19. Rye steep liquor, 1:2 + 0.5 per cent dextrose	14.60	11	2.57	25.7
20. Rye steep liquor, 1:4 + 0.5 per cent dextrose	9.70	11	1.70	34.1
21. Oats steep liquor, 1:2 + 0.5 per cent dextrose	7.72	11	1.26	12.6
22. Oats steep liquor, 1:4 + 0.5 per cent dextrose	6.40	11	0.68	13.6
23. Wheat steep liquor, 1:2 + 0.5 per cent dextrose	10.50	11	1.65	16.5
24. Wheat steep liquor, 1:4 + 0.5 per cent dextrose	8.85	11	0.86	17.3
25. Extract from malted rye, 1:25 + 0.5 per cent dextrose	15.00	11	2.53	56.0
26. Extract from malted rye, 1:30 + 0.5 per cent dextrose	13.20	11	1.69	44.5

a Media were distributed in quantities of 150 ml. per 2-l. Erlenmeyer flask and 200 ml. per 3-l. Fernbach flask.

b All quantitative data represent mean figures obtained from at least 4 replicates harvested separately.

c Raw material: dry, germinated, malted, or nongerminated cereal grains and dextrose.

d Dilution factors: 1:5, 1:25, or similar, means that 1 part of dry raw material, or stock solution, was extracted with 4 or 24 parts by weight of water.

e Steep liquor, 100 kg. of grain and 425 l. water steeped at 100° C.: corn, 3 hours; sorghum, 45 min.; wheat, 30 min.; rye, 30 min.; oats, 45 min.

Addition of dextrose increased yields, and excellent results were obtained in the media to which 0.5 to 1.0 per cent dextrose was added. In table 6 are given representative data on the growth and yield of *Phytophthora infestans* in liquid media.

The yield, on the basis of fungus produced per l. of medium or per kg. of raw material used, varied greatly. All media which yielded more than 1 gm. of dry mycelium per l. consisted of extracts of normal or malted grains. Best of these were the extracts prepared from rye. Rye steep liquor produced the highest yield per l. of medium, consisting of 2.57 gm. of mycelium, and the extract of malted rye produced the highest yield per kg. of raw material. Therefore, in subsequent experiments extracts from malted rye were used frequently. The addition of 0.002 per cent of $FeCl_3$ increased the yield of mycelium up to 17 per cent.

In order to establish the rate of development of the fungus in liquid media and the time required for producing the optimal yield, a number of 2-liter Erlenmeyer flasks, each containing 100 ml. of malted-rye extract plus 0.5 per cent dextrose, were inoculated and incubated. Three to six flasks were removed periodically for determination of yield. The results presented in table 7 were obtained from two independent experiments.

TABLE 7.—*Rate of growth of Phytophthora infestans in liquid medium*

	Age of culture (days)											
	3	5	6	8	10	12	15	16	17	20	26	33
Yield of mycelium (gm./l.)	0.03	0.21	0.40	0.90	1.33	1.73	2.24	2.25	2.24	2.20	2.13	1.91

Maximum yields were obtained within 16 days; the range was 15 to 20 days, depending upon the rate of growth within different cultures. When the cultures reached the maximum yield, a slow decline occurred that could be explained by autolysis of the fungus cells.

The addition of colloidal matter to liquid media has been found by numerous investigators to stimulate the growth of microorganisms. An inquiry was made into the possible effect of agar on the growth of *Phytophthora infestans* in liquid culture. It was necessary to determine the effect of hot-water extraction upon the weight of mycelium, since such treatment would be necessary to remove the agar. The weight of the fungal mat from cultures steamed and rinsed with hot water was 47 per cent lower than that of the cultures harvested in the usual way. Following these preliminary studies, an experiment was made to ascertain the influence of agar on the yield of mycelium from liquid media. The medium used was a 1:25 extract from malted rye, plus 0.5 per cent dextrose. The medium was adjusted to pH 6.5. Agar was added at the rates of 0.10, 0.25, and 0.50 per cent, and the medium without agar was used for the control. These media were distributed in 100-gm. quantities into 2-liter, tared, Erlenmeyer flasks, sterilized, and inoculated with 1 ml. of sporangial suspension. After 12 days'

incubation, the flasks were weighed again and the weight made up to 100 gm. with distilled water. The cultures were steamed for 10 minutes, filtered while hot, and the fungal mat, which was collected on tared filter paper, was rinsed with boiling water to remove all of the agar.

Control cultures on medium without agar were harvested in the same way. After the fungal mats were weighed, the yield figures were increased by 47 per cent to make them comparable with other experiments in which the fungal mats were neither steamed nor rinsed with boiling water. The results indicated that the addition to the medium of colloidal material in the form of agar increased the yield of mycelium by 37 to 41 per cent. There was very little difference in yield from media containing various concentrations of agar. The average yield of dry mycelium was 3.62 gm. per l. of medium and 80 gm. per kg. of raw materials.

Inasmuch as the extract from malted rye was selected as the best medium,

TABLE 8.—*The utilization of the extract from malted rye*[a]

Determinations[b]	Noninoculated control	Inoculated media		
Quantity of medium per flask (gm.)	100.00	100.00	100.00	100.00
Loss due to sterilization and incubation, per flask (gm.)	13.35	12.58	12.50	12.20
Yield of fungus, dry weight (gm./l.)	0.00	2.19	2.25	1.80
Soluble solids used to produce 1 gm. of mycelium (gm.)	0.00	1.82	1.85	1.81
Soluble solids remaining in medium at time of harvest (gm./l.)	12.08	8.11	7.92	6.82
Total sugar as glucose (gm./l.)	9.30	4.60	4.30	....
Glucose (gm./l.)	6.20	1.90	1.70	....

[a] All figures represent averages from at least 5 replicates.
[b] The medium was prepared quantitatively from extract from malted rye, 1:25 + 0.5 per cent dextrose. The medium was dispensed in 2-liter Erlenmeyer flasks, sterilized, inoculated, and incubated for 13 days at about 20° C.

more detailed tests were made to estimate the rate of the utilization of the medium by this organism (Table 8). Over 70 per cent of the total solids present were accounted for after hydrolysis as glucose. About 50 per cent of the total sugar and about 70 per cent of the sugar determined prior to hydrolysis as glucose was utilized by the fungus.

The growth of many fungi in liquid culture is increased by mechanical agitation or forced aeration. The effects of these conditions, and the depth of the culture medium, on the growth of *Phytophthora infestans* were tested. In experiments on the effects of agitation and depth of the medium, a 1:20 extract of germinated wheat plus 0.5 per cent dextrose was used in 2-liter Erlenmeyer flasks at depths of 15, 25, and 35 mm. Representative data are given in table 9. The best growth under conditions of these experiments was obtained in the undisturbed, shallow cultures. Aerial mycelium was abundant in these cultures and absent in all other. In experiments on the effect of forced aeration, *P. infestans* was grown in 4-liter Pyrex serum bot-

tles containing 2 liters of a 1 : 25 extract of malted rye plus 0.5 per cent dextrose medium. The cultures were aerated by bubbling during the entire 15-day incubation period. The average yield per liter for 9 replicates was 1.68 gm. while that in the unaerated controls was hardly perceptible. Thus forced aeration increased yields in these deeper cultures, but still did not equal the gm. per l. yield obtained in the shallow, undisturbed cultures (Table 9).

Longevity and Storage

Attempts were made to increase the longevity of *Phytophthora infestans* mycelium during storage by partial dehydration of the fungal mat harvested from liquid media. Individual mycelial mats, harvested from cultures in 250-ml. Erlenmeyer flasks, were rinsed in sterile distilled water, drained, folded into a reniform mass and suspended on filter paper supports over the surface of sulphuric acid of concentrations to give relative humidities of

TABLE 9.—*The influence of the depth of medium and agitation upon yield*[a]

Treatment	Depth of medium	Yield per	
		liter of medium	kg. of raw material
	mm.	*Grams*	*Grams*
Agitated once a day	15	0.75	13.68
Undisturbed	15	2.74	49.80
Do	25	0.94	17.10
Do	35	0.33	5.93

[a] The cultures were incubated for 12 days at about 20° C.

80.5 to 98.2 per cent (20). Samples were removed and the viability of the mycelium tested by transferring it to slants of rye-malt-extract plus dextrose agar. The mycelium was viable for more than 23 days at 98.2 per cent relative humidity, for 23 days at 96.2 per cent, for 9 days at 92.0 per cent, for 6 days at 84.0 per cent, and for 2 days at 80.5 per cent. Thus partial dehydration of *P. infestans* mycelium impaired rather than increased its longevity in storage.

Observations of de Bruyn (8) indicated that *Phytophthora infestans* survived up to four years in manure or soil. Therefore, tests were carried out on the longevity of the fungal mats stored in distilled water, media in which the fungus was cultivated, and in sterile soil. Individual fungal mats from malted-rye-extract plus dextrose medium were deposited in half-pint fruit jars, each containing one of the three substrates. "Soil" was prepared by mixing 10 parts of fine, sifted garden-soil with 1 part of powdered peat; it was saturated with water. The mycelium was buried in the soil. All jars were stored at 5° to 10° C. Periodically, mycelium was removed and transferred to slants of rye-malt-extract plus dextrose agar to test its viability. The fungus was viable up to 146 days in soil or distilled water, but in the culture media viability was greatly reduced. Macroscopic observations indicated that the fungus grew slowly during storage.

In another test the fungus was stored under similar conditions but in water containing 1, 5, 10, or 40 per cent dextrose. In 40 per cent dextrose the fungus died within 2 days but in all other concentrations it was still viable after 52 days.

DISCUSSION AND SUMMARY

The purpose of the experiments reported was to develop simple and reliable methods for the production of sporangia and mycelium of the potato-late-blight fungus and to prolong its viability in storage. On solid, well-aerated, porous media consisting of cereal grains and peanut hulls saturated with water, the fungus grew well and produced sporangia abundantly. When the cereal grains were replaced with certain legume seeds, such as navy bean, growth was even more abundant but sporangia were produced in smaller numbers.

In liquid media growth was also excellent but the number of sporangia produced was insignificant. Liquid media were only adapted to the production of the fungus in its mycelial form.

Artificial aeration of deep cultures of liquid media was essential for good growth. Where the media were in shallow layers, best growth was obtained in undisturbed cultures.

Under optimal conditions, the maximal yield of sporangia was obtained after 8 to 10 days' incubation. Sporangia could be harvested by washing them away from the medium with sterile water. About 90 per cent of freshly harvested sporangia were viable. They were in general short-lived, but the longevity could be somewhat increased by maintaining them in the original undisturbed culture or by storing them in a 10 to 20 per cent solution of dextrose at temperatures slightly above freezing.

Mycelium could be grown easily in shallow culture on media consisting of the extracts from malted cereal grains, particularly rye with added dextrose. In such media, yields amounting to 2.5 to 3.0 gm. of dry mycelium per l. of medium or more than 50 gm. of dry mycelium per each kg. of dry raw materials used in the preparation of the media were obtained. Addition of small amounts of agar to liquid media increased the yield considerably.

Mycelium survived longer in storage than sporangia. If stored at low temperatures in water or wet soil, it remained viable at least 146 days.

CAMP DETRICK,

FREDERICK, MARYLAND.

LITERATURE CITED

1. CLINTON, G. P. Downy mildew, or blight, *Phytophthora infestans* (Mont.) De Bary of potatoes II. Conn. Exp. Stat. Ann. Rept. 1905: 304–330. 1906.
2. ————. Report of the botanist for 1908. IV. Artificial cultures of *Phytophthora* with special reference to oospores. Conn. Exp. Stat. Rept. 1907–1908: 891–907. 1908.
3. ————. Oospores of potato blight, *Phytophthora infestans.* Conn. Exp. Stat. Rept. 1909–1910: 753–774. 1911.

4. CROSIER, W.   Culture of *Phytophthora infestans*.   Phytopath. **23**: 713–720. 1933.

5. —————.   Studies in the biology of *Phytophthora infestans* (Mont.) De Bary. Cornell Univ. Agr. Exp. Stat. Mem. 155. 1934.

6. DE BARY, A.   Researches into the nature of the potato fungus, *Phytophthora infestans*.   Jour. Agric. Soc. [England], Ser. 2, **12**: 239–269. 1876.

7. DE BRUYN, HELENA L. G.   The saprophytic life of *Phytophthora* in the soil. Meded. Landbou. Wageningen. **24**(4): 1–38. 1922.

8. —————.   The overwintering of *Phytophthora infestans* (Mont.) De Bary. Phytopath. **16**: 121–140. 1926.

9. FOSTER, J. W., L. E. MCDANIEL, H. B. WOODRUFF, and J. L. STOKES.   Microbiological aspects of penicillin.   V.   Conidiospore formation in submerged cultures of *Penicillium notatum*.   Jour. Bact. **50**: 365–368. 1945.

10. JONES, L. R., N. J. GIDDINGS, and B. F. LUTMAN.   Investigations of the potato fungus *Phytophthora infestans*.   U. S. Dept. Agr. Bur. Plant Ind. Bull. 245. 1912.

11. KOSSOWICZ, A.   Die Zersetzung von Harnstoff, Harnsäure, Hippursäure und Glykokol durch Schimmelpilze.   Zeitschr. f. Gärungsphysiol. **1**: 60–62. 1912.

12. —————.   Über das Verhalten einiger Schimmelpilze zu Kalkstickstoff.   Zeitschr. f. Gärungsphysiol. **1**: 124–125. 1912.

13. —————.   Die enzymatische Natur der Harnsäure- und Hippursäure-Gärung. Zeitschr. f. Gärungsphysiol. **1**: 317–319. 1912.

14. —————.   Die Zersetzung von Harnstoff, Harnsäure, Hippursäure und Glykokol durch Schimmelpilze.   Zeitschr. f. Gärungsphysiol. **2**: 51–55. 1913.

15. —————.   Nitritassimilation durch Schimmelpilze.   Zeitschr. f. Gärungsphysiol. **2**: 55–58. 1913.

16. —————.   Die Zersetzung von Harnstoff, Harnsäure, Hippursäure und Glykokol durch Schimmelpilze.   Zeitschr. f. Gärungsphysiol. **2**: 81–83. 1913.

17. —————.   Die Assimilation von Guanin und Guanidin durch Schimmelpilze. Zeitschr. f. Gärungsphysiol. **2**: 84–86. 1913.

18. —————.   Über das Verhalten einiger Schimmelpilze zu Kalkstickstoff.   Zeitschr. f. Gärungsphysiol. **2**: 154–157. 1913.

19. MATRUCHOT, L. et M. MOLLIARD.   Sur le *Phytophthora infestans*.   Ann. Mycol. **1**: 540–543. 1903.

20. STEVENS, N. E.   A method for studying the humidity relations of fungi in culture. Phytopath. **6**: 428–432. 1916.

21. UPPAL, B. N.   Spore germination of *Phytophthora infestans*.   (Abstr.) Phytopath. **14**: 32. 1924.

22. VOWINCKEL, O.   Die Anfälligkeit deutscher Kartoffelsorten gegenüber *Phytophthora infestans* (Mont.) De Bary, unter besonderer Berücksichtigung der Untersuchungsmethoden.   Arb. Biol. Reichsanst. Land- u. Forstw. **14**: 488–641. 1926.

THE EFFECT OF SEED TREATMENTS, COMMERCIAL FERTILIZERS, AND MINOR ELEMENTS ON ROOT ROT, STAND, AND YIELD OF POD PEAS[1]

J. L. FORSBERG[2] AND A. M. BINKLEY[3]

Each year root-rot fungi cause losses of stand and reduce yields of pod peas in Colorado. The data presented in this paper are confined to a series of tests in the field and greenhouse to determine the effectiveness of seed treatments, commercial fertilizers, and minor elements in reducing losses due to root rot and seed rot.

SEED TREATMENTS

Data were obtained from three seed-treatment plots in 1944. One plot of the pea variety Laxtonian was planted at Rocky Ford and one plot each of Little Marvel and Rogers' 95 at Fort Collins. All plots were of the Latin square design using six treatments and six replications. Each replication consisted of two rows with 50 seeds per row. Treatments used were Spergon (98 per cent tetrachloroparabenzoquinone) Arasan (50 per cent tetramethylthiuramdisulfide), and Yellow Cuprocide (93 per cent yellow cuprous oxide), each at the rate of 2 ounces per bushel of seed, and New Improved Ceresan (5 per cent ethyl mercury phosphate) and Du Pont 1452–F (7.7 per cent ethyl mercury p-toluene sulfonanilide) at the rate of 1 ounce per bushel. Emergence counts were made on all plots, and yield records were obtained from the two plots at Fort Collins. All data were analyzed by the analysis of variance method.

Emergence counts are summarized in table 1. In the Laxtonian plot at Rocky Ford, New Improved Ceresan, Du Pont 1452–F, Spergon, and Yellow Cuprocide treatments resulted in stands which were significantly better than the nontreated. Treatments with New Improved Ceresan, Du Pont 1452–F, and Arasan resulted in significant stand increases in the Little Marvel plot. Increases resulting from Spergon and Cuprocide treatments approach the 5 per cent level of significance. All treatments in the Rogers' 95 plot resulted in highly significant stand increases.

The yield records in table 2 show that significant increases were obtained from Arasan, New Improved Ceresan, Spergon, and Du Pont 1452–F in the test with Little Marvel peas. No significant yield increases were obtained on the Rogers' 95 plot.

The results agree in general with those obtained in the 1943 tests.[4] Du

TABLE 1.—*Effect of pea seed treatment on emergence*

Treatment	Mean number of plants emerged from 100 seed		
	Laxtonian	Rogers' 95	Little Marvel
Spergon	90.83	79.33	67.33
Arasan	87.67	79.67	68.16
Cuprocide	91.17	79.83	67.33
New Improved Ceresan	92.00	87.50	71.33
Du Pont 1452–F	93.00	84.17	71.83
Nontreated	87.17	60.67	60.33
Difference required for significance:			
5 per cent level	3.21	4.96	7.53
1 per cent level	4.37	6.77	10.27

Pont 1452–F, which was not used in 1943, showed up well in the 1944 trials. All the treatments used gave some increases in stand. New Improved Ceresan and Du Pont 1452–F gave the best stands in all three plots. No correlation between stands and yields was determined.

SEED TREATMENTS AND FERTILIZERS

In an attempt to determine whether a combination of seed treatment and fertilizer had any effect on stand and survival of plants, a plot in which seed treated with Spergon, New Improved Ceresan, and Yellow Cuprocide and nontreated seed were planted in combination with seven fertilizers and a nonfertilized check. This plot was located in the San Luis Valley on variable, rocky-gravelly soil which was sub-irrigated during the growing season. The fertilizer ratios used were 5–0–0, 0–30–0, 0–0–4, 5–30–0, 5–0–4, 0–30–5, and 5–30–4. Nitrogen was added as ammonium sulfate, P_2O_5 as treble superphosphate, and K_2O as muriate of potash. The fertilizer was drilled in so it was placed below the seed. The rate of application was 200 lb. per acre. Plots were single rows, 20 feet long with 102 seeds of Rogers' 95 variety planted per row. Each treatment was replicated five times on a factorial design basis. Stand counts were made one month after planting. The numbers of living plants were counted at picking time and yields of pod peas were recorded.

TABLE 2.—*Effect of seed treatment on yield of peas*

Treatment	Mean yield in ounces of pot peas per plot	
	Rogers' 95	Little Marvel
Spergon	191.3	101.7
Arasan	183.0	111.0
Cuprocide	178.2	93.5
New Improved Ceresan	189.8	106.0
Du Pont 1452–F	186.7	101.5
Nontreated	171.9	90.8
Difference required for significance:		
5 per cent level	27.7	
1 per cent level	9.3	12.7

TABLE 3.—*Effects of seed treatments and fertilizers on emergence of peas*

Fertilizer ratio	Mean number of plants emerged per row				
	Nontreated	Cuprocide	New Improved Ceresan	Spergon	Average
0–0–0	28.4	53.4	76.6	61.2	54.9
5–0–0	19.8	52.0	71.6	59.6	50.6
0–30–0	25.8	43.4	80.6	67.0	54.2
0–0–4	33.2	49.0	73.2	65.4	55.2
5–30–0	27.8	48.6	66.8	48.6	47.9
5–0–4	21.0	43.2	75.6	63.0	50.7
0–30–4	21.2	39.2	72.0	59.4	47.9
5–30–4	33.6	50.0	68.0	58.8	52.9
Average	26.4	47.4	73.1	60.4	....
Difference required for significance:			5 per cent level	1 per cent level	
Between seed treatments			6.6	8.7	
Between fertilizers			N.S.	N.S.	
Interaction: seed treatments × fertilizers			N.S.	N.S.	

As shown in table 3, stand increases from all seed treatments were highly significant. New Improved Ceresan gave decidedly the best stand and was followed by Spergon and Yellow Cuprocide. Differences between seed treatments were highly significant. There were no significant differences between fertilizers.

Counts of living plants at harvest time showed that the numbers surviving in the treated lots were significantly greater than those from nontreated seed. Differences between seed treatments were not significant. None of the fertilizers resulted in significantly greater survival of plants. No significant interaction between seed treatments and fertilizers was obtained.

All plots from treated seed produced higher yields of pod peas than the plots from nontreated seed. None of the fertilizer applications resulted in increased yields (Table 4).

TABLE 4.—*Effects of seed treatments and fertilizers on survival of pea plants*

Fertilizer ratio	Mean number of living plants at harvest time				
	Nontreated	Cuprocide	New Improved Ceresan	Spergon	Average
0–0–0	16.0	28.4	18.6	33.2	24.1
5–0–0	13.8	32.8	28.6	27.6	25.7
0–30–0	19.8	21.6	33.8	32.8	27.0
0–0–4	15.8	22.2	26.2	36.4	25.2
5–30–0	11.8	28.0	25.8	34.8	25.1
5–0–4	11.0	23.0	30.0	29.6	23.4
0–30–4	11.6	20.2	31.6	21.6	21.3
5–30–4	14.8	18.4	18.2	30.8	20.6
Average	14.3	24.3	26.6	30.9	....
Difference required for significance:			5 per cent level	1 per cent level	
Between seed treatments			5.8	7.7	
Between fertilizers			N.S.	N.S.	
Interaction: seed treatments × fertilizers			N.S.	N.S.	

MINOR ELEMENTS

An experimental plot designed to determine whether the elements Cu, Fe, Zn, and Mn had any effect on root rot was planted in the San Luis Valley. Each of the four elements, applied in the form of its respective sulfate, was used alone and in all possible combinations. Applications at the rate of 25 lb. per acre were made by drilling so the chemicals were placed in bands on each side of the row. Single 20-foot rows were used with a buffer row between plots. A randomized block with five replications of each treatment was used in the test. Rows were 34 inches apart. All seed was treated with Spergon. Records were made of emergence, number of living plants at harvest time, and yield of pod peas. No significant differences were obtained, because the values required for significance were too high on account of soil or other variations. It was noted, however, that the plants in some of the rows on which Mn was applied remained greener longer than the plants in adjacent nontreated rows.

THE EFFECT OF SEED TREATMENTS IN DIFFERENT SOILS

Two sets of experiments were run in the greenhouse in an attempt to determine why the results of seed treatment tests in the 1943 field trials were inconsistent. In the first experiment the effects of New Improved Ceresan and Spergon on plant emergence and survival were tested in nine different soils, seven of which had been collected in pea fields. Ten seeds of the Wisconsin Sweet variety were planted in each 6-inch pot and the pots were arranged in a randomized block of four replications. Emergence counts were made two weeks after planting, and living plants were counted again at the end of two months.

TABLE 5.—*Effect of pea seed treatment in different soils on emergence and survival of pea seedlings*

Source of soil[a]	Mean numbers of plants emerged (Emer.) and number of plants surviving (Surv.) for 2 months					
	Nontreated		New Improved Ceresan		Spergon	
	Emer.	Surv.	Emer.	Surv.	Emer.	Surv.
Fort Collins	·5.0	3.5	8.5	7.0	6.7	5.0
Fort Collins	5.5	5.5	9.2	7.7	7.7	·6.0
Center	7.0	6.2	7.2	6.7	9.0	9.0
Sanford	5.0	4.2	7.5	6.2	8.2	7.0
Del Norte	6.2	5.2	8.0	5.5	9.0	8.5
La Jara	4.0	3.0	6.5	5.0	7.5	7.5
Monte Vista	7.5	5.7	8.2	7.5	8.5	7.2
Fort Collins (foothills)	7.0	7.0	7.2	6.0	8.7	8.2
River sand	9.2	8.5	9.2	9.0	8.5	7.0

Difference required for significance:		Emergence	Survival
5 per cent level		1.91	2.33
1 per cent level		2.53	3.08

[a] Soils taken from pea fields except for the Fort Collins foothills soil, and the river sand.

The effect of seed treatments in different soils is summarized in table 5. In both soil samples from Fort Collins, seed treated with New Improved Ceresan produced more plants than nontreated seed. The difference was highly significant. The increase obtained from Spergon was significant at the 5 per cent level. When Spergon-treated seed was planted in soils from Sanford, Del Norte, and La Jara the increase in emergence over that obtained from nontreated seed was highly significant. In two of these soils New Improved Ceresan gave an increase which was significant at the 5 per cent level. No significant differences were obtained on soils from Center, Monte Vista, Fort Collins foothills, and river sand.

The mean number of living plants two months after planting show the same general trends as the emergence counts, but the difference required for significance is greater than that required in the emergence counts. This indicates that factors other than seed treatment influenced the number of plants remaining alive.

THE EFFECT OF SEED TREATMENTS ON DIFFERENT FUNGI

In the second experiment four fungicides, Spergon, Arasan, Yellow Cuprocide, and New Improved Ceresan, were tested for effectiveness against four fungi isolated from diseased peas, *Rhizoctonia solani* Kühn., *Pythium ultimum* Trow., *Ascochyta pinodella* Jones, and *Fusarium solani* (Mart.) v. *martii* (App. et Wr.) Wr. f. 2 Snyd. Pea varieties used were Laxtonian,

TABLE 6.—*Effect of seed treatment on emergence of pea seedlings in soils inoculated with different fungi*

Variety and inoculation	Mean number of plants emerged per pot				
	Nontreated	Spergon	Arasan	Cuprocide	New Improved Ceresan
Rogers' 95					
No fungus	8.8	8.6	9.6	8.2	9.2
Rhizoctonia	0.0	6.6	4 6	2.2	5.6
Pythium	0.0	0 0	0.2	0.2	0.0
Ascochyta	5.6	8.6	8.2	7.4	6.2
Fusarium	7.6	7.4	7.6	6.2	7.8
Little Marvel					
No fungus	8.6	8.2	9.0	9.4	8.6
Rhizoctonia	0.0	7.0	5.0	0.6	0.2
Pythium	0.0	4.4	2.0	1.4	0.0
Ascochyta	8.6	7.8	8.8	8.2	7.8
Fusarium	7.8	8.8	6.8	8.6	7.8
Laxtonian					
No fungus	4 3	4 5		4.8	4.7
Rhizoctonia	0.3	4.1		3.2	4.2
Pythium	0.0	0.0		0.0	0.0
Fusarium	0.3	0.3		0.0	0.6

Difference required for significance	5 per cent level	1 per cent level
Rogers' 95	2.02	2.68
Little Marvel	1.41	1.86
Laxtonian	0.53	0.70

a Five seeds planted per pot.

Little Marvel, and Rogers' 95. Pure cultures of each fungus were grown for two weeks on autoclaved oats in 250-ml. Erlenmeyer flasks. The contents of one flask were mixed with steamed soil for two 6-inch pots, and the mixture was allowed to stand in the pots for one week. Ten seeds were planted in each pot. The pots were arranged in an equalized block design of five replications. They were watered as needed to keep the moisture content of the soil favorable for plant growth. Emergence counts were made two weeks after planting.

Emergence counts obtained in these experiments are summarized in table 6. No significant increase in emergence was obtained from any seed treatment when *Fusarium solani* var. *martii* f. 2 was in the soil. Emergence
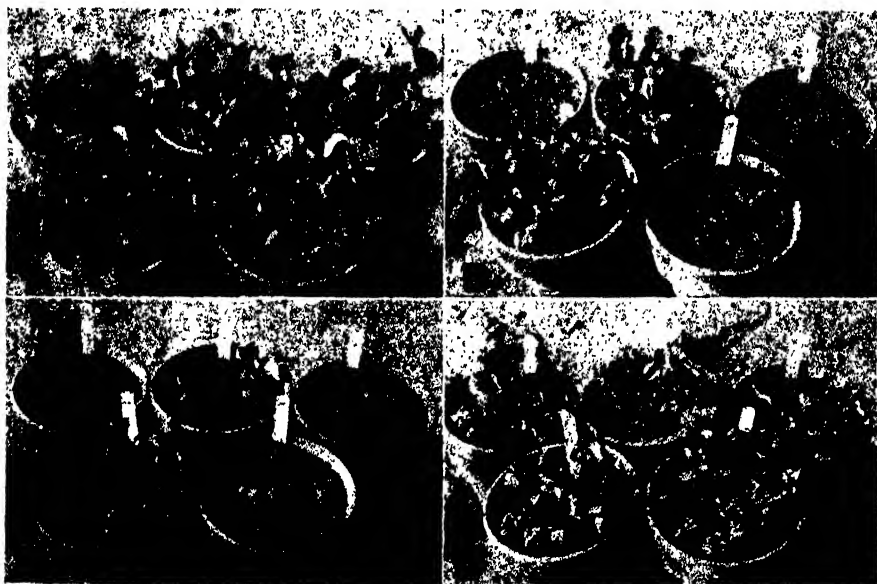
FIG. 1. Little Marvel peas in steamed soil without fungus inoculation (A), with *Rhizoctonia solani* (B), with *Pythium ultimum* (C), with *Fusarium solani* var. *martii* (D). In each series reading from left to right. Back row, left, untreated seed; center, Spergon-treated seed; right, Arasan-treated seed. Front row, left, Cuprocide-treated seed; right, New Improved Ceresan-treated seed.

in the Laxtonian variety was greatly reduced by this fungus; emergence of Rogers' 95 was slightly reduced, but emergence of Little Marvel was not significantly affected.

Spergon gave a significant increase in emergence in all varieties when *Rhizoctonia solani* was contained in the soil. Arasan was also effective in the two tests in which it was used on this fungus. New Improved Ceresan gave increased emergence with Laxtonian and Rogers' 95. Cuprocide was only slightly effective against *Rhizoctonia*.

Spergon, Arason, and Cuprocide gave a slight degree of control of *Pythium ultimum* on Little Marvel, but no fungicide was effective against this fungus in the other two tests.

Spergon and Arasan were effective against *Ascochyta pinodella* in the Rogers' 95 test, but treatments were not significantly better than the non-treatment in Little Marvel.

DISCUSSION

These experiments show that seed treatments are beneficial in increasing the emergence of pod peas. Fertilizers were not beneficial in these tests. Attack by root rotting organisms after the plants have emerged is not controlled by seed treatment, but the protection offered against pre-emergence attack is often great enough to result in increased yields. The chemicals used as seed treatments vary in their effectiveness against different organisms. Pea varieties vary in their reactions, but the type and amount of fungus in the soil is probably the most important factor affecting emergence of pea seedlings.

SUMMARY

Pea seed treatment with New Improved Ceresan, Arasan, Spergon, Yellow Cuprocide, and Du Pont 1452–F generally resulted in increased stands. Combinations of fertilizers with seed treatments did not increase the effectiveness of the seed treatments. Addition of Cu, Fe, Zn, and Mn did not increase stands or yields. Effectiveness of seed treatment varied in different soils, against different fungi, and on different pea varieties.

COLORADO AGRICULTURAL EXPERIMENT STATION,
FORT COLLINS, COLORADO.

CONCEALED DAMAGE OF PEANUTS IN ALABAMA[1]

C O Y T   W I L S O N

INTRODUCTION

Concealed damage is the name used in the peanut trade to designate a type of damage that is not visible until the seed is broken open. It constitutes the greatest obstacle the sheller must overcome in supplying the trade with good quality peanuts. Shrivels, splits, and foreign matter can be removed by screening or winnowing, and visibly damaged seeds can be removed on the picking belt. On the other hand, seeds with concealed damage cannot be separated from sound ones by any method in use at present. The present procedure is to sample and grade the peanuts as they are delivered to the shelling plant and segregate those lots that contain appreciable amounts of this type of damage. This requires additional storage space and involves extra labor.

In addition to expense and inconvenience to shellers and processors, there is economic loss to the grower. Under prevailing market regulations, the price received by the grower is determined by the percentage of sound mature seeds that can be shelled from a sample and by the amount of damage. If damage exceeds 2 per cent a penalty is imposed. Since each damaged seed might have been sound, losses to the grower are pyramided. He loses what he would have received had the damage failed to develop and he pays a penalty because it did.

Concealed damage is more prevalent in peanuts of the runner type than in Spanish peanuts. Since about 95 per cent of Alabama's peanut acreage is planted to runner peanuts, this disease is of great importance to the farmers of southeastern Alabama. In 1945 losses were exceptionally heavy, approximately 2¼ million dollars in Alabama, and many growers suffered penalties of $25 to $75 a ton.

Concealed damage has been known for several years. Peanut processors and shellers in southern Alabama and Georgia report that some confectioners and candy manufacturers discontinued the use of runner peanuts as early as 1930 because of its prevalence. Little concern was evidenced, however, until present marketing regulations became effective January 1, 1944. At that time there was relatively little information available on the cause, epiphytology, or control of the disease. In July, 1944, Higgins (2) reported that *Rhizoctonia, Sclerotium bataticola, Pencillium,* and *Rhizopus* had been isolated from damaged seeds and that the incidence of disease could be materially reduced by harvesting methods that would promote rapid curing. In the fall of that year, work was begun at the Alabama Agricultural Experi-

ment Station to determine the cause of concealed damage, and the factors that affect its development, so that control measures could be studied on a sound experimental basis.

METHODS AND MATERIALS

The studies on the etiology, epiphytology, and control of concealed damage have consisted of field surveys and observations, and laboratory experiments.

The amount of damage was determined as follows: Samples of either 300 or 500 gm. of unshelled peanuts were screened to remove dirt and small rocks, and all other foreign matter was removed by picking. The sample was shelled, and the shriveled seeds were removed by screening. Visibly damaged seeds were next removed. The remaining seeds were cracked open and those having damaged cotyledons were removed and weighed. This damage, expressed as a percentage of the original unshelled sample, was recorded as concealed damage.

In isolating from the damaged nuts, four seeds were placed in each Petri dish, in order to conserve time and space. Cultures were transferred to agar slants for identification. With such a system, cultures rarely are obtained from all of the seeds plated. Seeds that are slow in yielding cultures frequently are over-run by the cultures from other seeds.

Additional methods and materials are given where necessary.

THE DISEASE

Symptoms. Concealed damage first becomes evident as a slight discoloration on the inner face of the cotyledon. As the disease progresses, the discoloration changes from light yellow to dark yellow and eventually to dark purple. A dense mycelial mat, usually white, gray, or a very light purple, develops between the cotyledons. No fruiting structures have ever been observed on this mycelial mat. As internal discoloration becomes more pronounced, changes occur externally. The seed coat becomes shriveled, the seed loses its brittleness, and it finally develops a dark purple to black color. These changes are shown in figure 1. A strongly rancid or bitter taste develops before there are any external symptoms.

The mycelium develops in the cavity between the cotyledons, but no penetration of the host tissues by the hyphae has been observed in such seeds. Apparently breakdown of the host cells is accomplished by toxins that are secreted by the mycelium. In the later stages, when external symptoms are evident, hyphae can be found in and between the cells of the host tissue.[a]

[a] These symptoms apply to more than 95 per cent of the affected seeds collected in Alabama. Occasionally slightly shriveled seeds that appear to be sound on the outside possess an enlarged cavity between cotyledons. The inner faces of these cotyledons are sometimes slightly discolored but the trouble appears to be physiological. Another type of damage sometimes encountered seems to be a soft rot that begins in the "germ" of the seed. These two types of damage are relatively unimportant in Alabama and have been excluded from the discussion that follows.

Either one or both of the seeds within a pod may be damaged. There is no tendency for the disease to begin at either end of the pod.

Effect on Quality. There is relatively little information concerning the effect of concealed damage on the chemical composition of the seed. The data that are available deal with the properties of the oil from peanuts containing varying amounts of damage. Woodruff and Cecil (7) cured peanuts in 21 different ways and noted the effect of the method of curing on the quality of the seed and the properties of the oil. Their figures show that the percentage free fatty acids in the oil rises as the damage increases. There was no correlation between the percentage damage and the total oil, or the refractive index, viscosity, specific gravity, or light transmission of the oil.
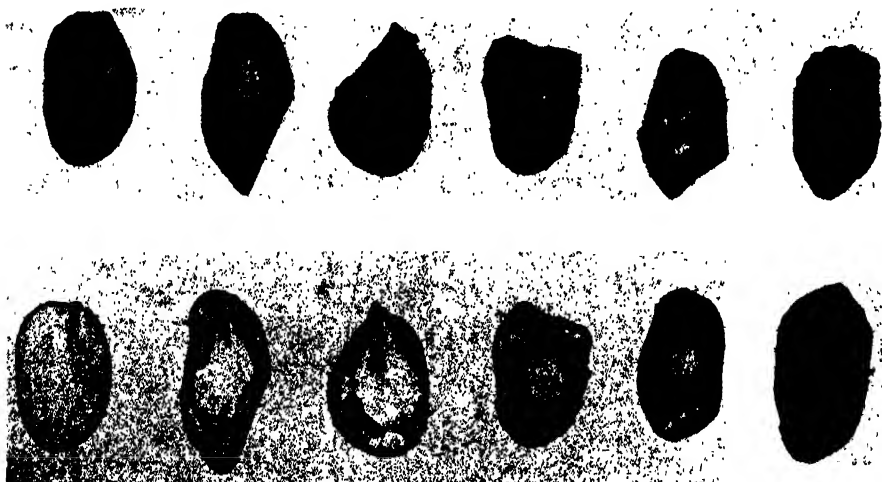
FIG. 1. Outside and inside views of peanuts with concealed damage. The seed at the left is not damaged. The other five seeds show varying degrees of damage. At the extreme right the damage is no longer concealed.

Stansbury, Guthrie, and Hopper (3), at the Southern Regional Research Laboratory in New Orleans, also found that the percentage of free fatty acids was higher in oil from samples containing damaged seeds. There was no correlation between the percentage damage and the total oil content, the nitrogen content, or the iodine number of the oil.

In order to establish the relationship between the percentage of damage and the percentage of free fatty acids, samples of peanuts were broken open and all damaged seeds removed. The sound seeds and the damaged seeds were ground and passed separately through a 16-mesh screen. From the meal obtained, 100-gm. samples containing definite proportions of damage were prepared. Samples containing 0, 2, 5, 10, 25, 50, and 100 per cent damage were used. The oil was expressed from these samples in a hydraulic press at a pressure of 4,500 pounds per square inch for 30 minutes. The percentage of free fatty acids was determined by titration with 0.1 N NaOH.

The average percentage of free fatty acids varied from 0.309 in the oil from undamaged peanuts to 15.341 in the oil from the sample in which all seeds were damaged. The relationship existing between the percentage damage and the percentage of free fatty acids in the oil approaches a straight line (Fig. 2).

Point of Origin. Field surveys were made in 1944 and in 1945 to determine, so far as possible, the conditions under which concealed damage develops.

Twenty samples of peanuts were collected from as many different fields before digging. From each sample a 500-gm. lot was shelled and graded.
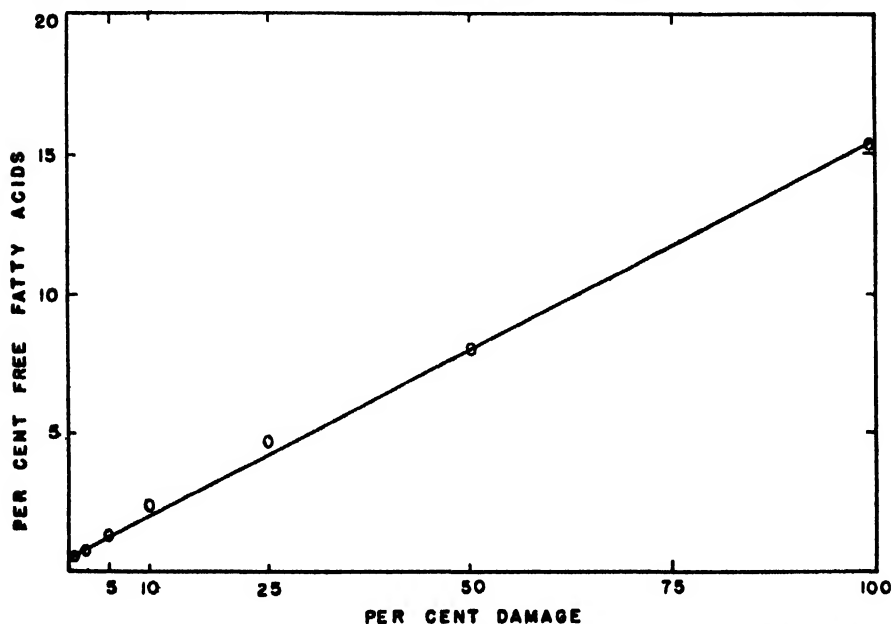
FIG. 2. The percentage of free fatty acids in the oil from peanuts containing various amounts of concealed damage.

In one lot, collected from an area of deep, coarse sand, 12 per cent damage was found; in another there was 3 per cent, and in another there was 2 per cent. In 10 other fields there was a trace (less than 0.5 per cent) of damage. In every instance the disease was still in its early stages. The cotyledons were distinctly yellow, and most of them had an unpleasant taste. No mycelial mats were found, but typical symptoms developed when samples were cured slowly. It is evident, therefore, that the disease can have its origin in the field.

These findings suggest the possibility that the damage that develops in the stack results from infections that occur in the field before the peanuts are dug. To check this possibility, green peanuts of the runner type were picked by hand, brought into the laboratory, graded, and found to contain 0.2 per cent damage. After being thoroughly mixed they were divided into

two lots.   One lot was spread on a hardware-cloth screen to cure rapidly;
the other lot was immersed in 0.5 per cent sodium hypochlorite solution for
two hours, then placed without washing in battery jars that had been washed
with 1 : 1,000 mercuric chloride solution.   These were covered with glass lids
without sealing, and placed on the laboratory shelves for one week.   The
peanuts were removed daily from the two battery jars, thoroughly mixed,
and returned to the jars.   This daily aeration prevented the formation and
retention of drops of free water on the peanut shells.   At the end of one
week the peanuts were transferred to new paper bags and stored for 35
days at room temperature, after which both lots were graded.   Those pea-
nuts cured rapidly on hardware-cloth screen contained only 0.5 per cent
damage, while those cured slowly after surface disinfection contained 12.0
per cent damage.   Thus, infection may occur before the peanuts are dug
and conditions prevailing during the curing process determine the extent
to which it develops.

TABLE 1.—*The relationship between the method of curing and the development of
concealed damage in runner peanuts*

Method of curing	Samples collected	Percentage of damage	
		Range	Average
	Number	*Per cent*	*Per cent*
Windrow	2	0.0– 0.5	0.25
Symmetrical stacks, well capped	7	0.5– 7.0	1.80
Rough or uncapped stacks	13	0.0–14.0	3.60
Cocks	7	1.0–10.0	5.70

Effect of Method of Curing[3] *on Incidence of Concealed Damage.*   In the
fall of 1944 after the peanuts had been dug, samples were collected from
different types of stacks, from cocks, and from windrows.   An effort was
made to obtain a representative sample from each stack or cock by selecting
peanuts from a number of points in and around the stack or cock and at
several points in the windrow.   Each sample consisted of about 2 lb. of
peanuts picked by hand.   Determinations of the amount of damage were
made on 500-gm. lots from each sample.   A summary of the findings is
in table 1.

Development of damage was associated with rate of curing.   Peanuts
cured rapidly in windrows contained· considerably less damage than those

[3] After peanuts are dug and the adhering soil removed by shaking, they are allowed
to cure until the moisture content of the seeds is reduced to about 10 per cent or less.
In dry weather some peanuts are cured loose in windrows.   The difficulty of moving dry
peanuts to the picker causes some growers to pile the peanuts into small to medium sized
heaps.   These are called cocks and usually are about 4 ft. in diameter and from 2 to 4 ft.
high.   The most common method of curing is by stacking.   A stack is formed by placing
wilted plants around an upright pole about 5 ft. high that has two cross arms 36 to 42 in.
long nailed to the pole at right angles to each other about 18 in. above the ground.   The
best stacks are about 4 ft. in diameter, are of symmetrical shape, and have the tip of the
stack pole covered with peanuts to provide a cap that sheds water.   However, stacks vary
considerably in size and shape in different fields depending upon the care with which they
were erected.

TABLE 2.—*Development of concealed damage in different parts of peanut stacks or cocks*

Sample No.	Method of curing	Amount of damage	
		Top—outside	Bottom—center
		Per cent	*Per cent*
1	Cock	1.0	11.0
2	Do	1.0	35.0
3	Do	0.0	6.0
4	Do	0.0	7.0
5	Do	0.0	18.0
6	Do	1.0	10.0
7	Do	1.0	8.0
8	Stack	1.0	3.0
9	Do	1.0	1.0
10	Do	0.0	20.0
11	Do	0.5	1.0

cured more slowly in stacks or cocks. This is further emphasized in table 2. Samples were collected from the top and outside and from the bottom and center of stacks and cocks, and the amount of damage was determined. With one exception there was considerably more damage on the inside where curing had been slow.

In 1945 green runner peanuts were picked by hand, thoroughly mixed, graded, and divided into four lots of 40 lb. each. At this time the peanuts contained 0.3 per cent damage. Each lot was cured in a commercially built home dehydrator cabinet from which the heating elements had been removed. The rate of curing was varied by disconnecting the circulating fans in some cabinets and by varying the size of the air vents in others. The results are in table 3. The fastest rate of curing was obtained in Cabinet No. 4, in which the moisture content dropped from 47.1 to 7.0 per cent within 5 days. The slowest rate of curing occurred in Cabinet No. 1, in which 19 days were required for the moisture to drop from 47.1 to 8.2 per cent. In Cabinets No. 2 and No. 3 the rates of curing were intermediate between that of No. 1 and that of No. 4.

However, concealed damage does not develop to any appreciable extent until the moisture content is reduced to about 35 per cent. The time required for completion of the curing process after this point is reached

TABLE 3.—*The rate of curing and the development of damage in runner peanuts cured in dehydrator cabinets*

Cabinet No.	Percentage of moisture								Percentage of damage at end of experiment
	At time of digging	After 1 day	After 2 days	After 3 days	After 5 days	After 7 days	After 11 days	After 19 days	
1	47.1	43.7	42.5	42.2	38.2	38.2	12.2	8.2	0.6
2	47.1	35.2	26.0	25.7	25.0	22.2	10.2	6.7	9.0
3	47.1	32.5	22.2	21.0	18.2	16.2	8.2	8.2	0.7
4	47.1	30.7	21.2	14.2	7.0	6.5	6.5	6.5	0.6

determines, to a large extent, the amount of damage that develops. Thus, in Cabinet No. 2, 10 days were required to reduce the moisture content from 35 to 10 per cent. These peanuts were 9.0 per cent damaged at the end of the experiment. The moisture content of the peanuts in Cabinet No. 1 remained above 38 per cent for 7 days, at which time the circulating fan was connected and the moisture content reduced to 12 per cent within 4 days. These peanuts had only 0.6 per cent damage even though the over-all rate of curing was about the same as in Cabinet No. 2.

Development of Concealed Damage in Peanuts Stored at Different Moisture Levels. Green peanuts were picked by hand, thoroughly mixed, and
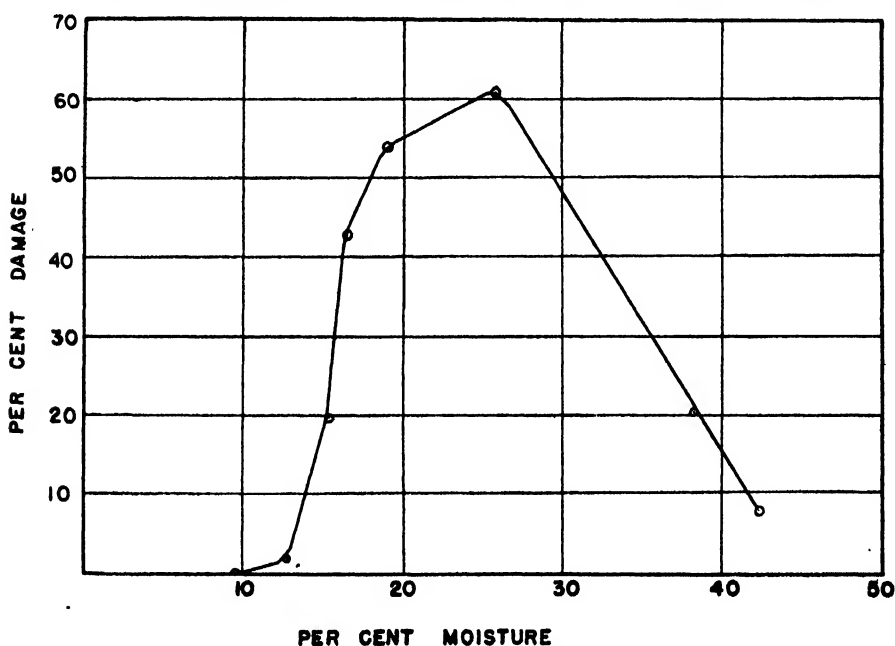
FIG. 3. Development of concealed damage in stored peanuts containing different amounts of moisture.

placed on a hardware-cloth screen in the greenhouse. Samples were removed at various times thereafter and stored in ½-gallon Mason jars. These were covered with glass lids but not sealed and stored in the laboratory at room temperature. The moisture content and the amount of damage were determined at the time the peanuts were put into the jars and again 68 days later when the peanuts were removed and graded. At the beginning of the experiment the peanuts contained 0.3 per cent damage. This initial 0.3 per cent was subtracted at the time the final notes were taken and the increase in damage recorded. The results are shown graphically in figure 3. There was no increase in damage in the peanuts stored at 9.5 per cent moisture or less. At 12.5 per cent moisture there was 2.2 per cent damage, an increase of 1.9 per cent. Above this moisture level the damage content rose

rapidly as the percentage moisture increased, reaching a total of 60.5 per cent when the peanuts were stored at a moisture level of 25 per cent. At higher moisture levels the percentage damage was less. There are no experimental data to explain the fact that green peanuts do not decay so rapidly as those that are partially cured. Where runner peanuts are "hogged-off" they sometimes remain in the soil for six weeks or two months after they are mature without appreciable seed decay. The lower temperatures that prevail in the soil probably prevent development of decay but the above data suggest that the high moisture content of the seeds also plays a rôle.

Effect of Soil Type, Fertilizer Practices, and Cropping System on Development of Concealed Damage. There appears to be no relationship between soil type or kind or amount of fertilizers used on the amount of damage that develops. No controlled experiments have been conducted, but concealed damage has been found in peanuts grown on light sandy soils, on sandy loam soils, on silt loam soils, and on clay soils. On some farms peanuts are planted without commercial fertilizer; on others various amounts of different kinds of fertilizers are used. Some growers use as little as 200 lb. an acre of 0–14–10 while others use as much as 400 lb. an acre of 3–8–5 with or without the addition of lime to the soil. Concealed damage may develop under any of these conditions. If these factors exert any influence at all on the development of concealed damage, their effect is very easily overshadowed by the weather conditions that prevail during the harvesting season.

Concealed damage seems to be slightly more prevalent in fields that are cropped to peanuts continuously. It is difficult to establish this fact by field surveys because of the variability of other factors. Nevertheless, the fields on which the higher percentages of concealed damage are found are usually those on which peanuts have been grown continuously for a number of years.

Isolations from Damaged Seeds. Preliminary studies in 1944 (5) revealed that, regardless of the medium used or the method of sterilization, the predominant organism isolated from seeds with concealed damage was *Diplodia theobromae* (Pat.) Nowell, an imperfect stage of *Physalospora rhodina* (B. and C.) Cke., according to Voorhees (4). Data obtained in 1945 and in 1946 confirm this conclusion. Most of the seeds were disinfected by dipping in silver nitrate, 1–1,000, for 2 to 3 minutes. They were transferred directly without washing to 1 per cent potato-dextrose agar acidified with lactic acid. A total of 2,729 seeds from 28 different samples have been plated. A total of 2,142 cultures of fungi were obtained, of which 1,869 or 87.2 per cent were *D. theobromae*. Various species of *Fusarium* ranked second with 116 cultures. *Sclerotium bataticola* was obtained from 28 seeds. *Sclerotium rolfsii* and *Rhizoctonia* were isolated from two seeds each. Miscellaneous fungi, including species of *Penicillium*, *Aspergillus*, and *Rhizopus* made up 125 cultures. Bacteria were isolated occasionally from seeds in the later stages of decay.

In order to determine the relationship between symptoms and fungi, 100

seeds with varying degrees of damage were selected. Each of these was numbered, and one half of each was plated. The other half was retained to determine fungi associated with symptoms. Of the 100 seeds plated, 90 yielded one or more fungi. *Diplodia theobromae* was isolated from 85 seeds and in 80 of these it was the only fungus obtained. *Sclerotium bataticola*, a species of *Fusarium*, *Rhizopus nigricans*, and unidentified fungi were isolated either alone or in combinations from 10 seeds. The 80 seeds yielding only *D. theobromae* showed symptoms of the early stages of concealed damage, while those that yielded other fungi or mixtures were in the advanced stages of decay. From these results it is evident that the primary invader and cause of concealed damage is *D. theobromae*. As the tissues break down, other fungi such as species of *Fusarium* spp., *S. bataticola*, and other soil inhabiting fungi enter as secondary invaders.

STUDIES ON CONTROL MEASURES

The results of the work on the relationship between the rate of curing and the development of concealed damage together with numerous field observations provide a basis for predicting practices that will reduce the amount of damage. These are (1) rapid curing methods, and (2) use of resistant varieties.

Since the disease develops most rapidly when peanuts are cured slowly, the moisture content should be reduced as rapidly as possible. Peanuts cure rapidly in windrows if there are not heavy and prolonged rains, but in Alabama a safer method is to cure the peanuts in small to medium sized stacks (6).

Spanish peanuts seldom contain more than 1 per cent concealed damage and often contain none at all. The reason for this has not been established. Spanish peanuts do not produce so much vine growth as runners and for this reason should cure faster. Another possible explanation lies in the nature of the seed coat. The seed of the runner peanut is covered with a fairly thick and tough skin, which effectively conceals the deterioration of the embryo through the early stages. The coat of the Spanish peanut is relatively thin and translucent, and any damage of the embryo soon becomes visible. It is also possible that the Spanish may be more resistant than the runner peanut.

Dixie Runner, a variety recently released by the Florida Agricultural Experiment Station, appears to have a great deal of resistance to concealed damage. Not more than 1 per cent concealed damage was found by Carver and Hull (1) in any of 30 samples of Dixie Runner grown in different parts of Florida. Ordinary Florida runners graded at the same time contained 8 to 20 per cent concealed damage.

Data collected in Alabama confirm the Florida results. In 1945 samples were collected from Dixie Runners stacked under different conditions and from cocks. There was less than 0.5 per cent concealed damage in each sample collected. Alabama runners cured under similar conditions contained 2 to 30 per cent concealed damage.

In 1946 approximately 25 lb. each of green Dixie Runners and Alabama runners were picked by hand. Both lots were placed in the same drying cabinet and cured slowly to promote the development of concealed damage. When the peanuts were graded 65 days later, the Dixie Runners contained only 1 per cent concealed damage, while the Alabama runners contained 6 per cent. Since the two varieties were grown on different fields these results if taken alone would not be conclusive.

Further evidence of the resistance of Dixie Runner was obtained as follows: The peanuts from a variety test, planted in duplicate, were harvested and cured in cocks on the ground. Both replicates of a given variety were mixed and placed in one cock. After 2 months, a 3-lb. sample was picked by hand from each variety. The ordinary Alabama runners contained 9.5 per cent concealed damage. The Dixie Runner contained only 1 per cent. Two other hybrid lines were intermediate between these.

The data taken collectively appear to be sufficient to prove that Dixie Runner is more resistant to concealed damage than the runners commonly grown in Alabama and Florida. They are not sufficient to establish definite numerical ratings of the resistance of the different varieties.

DISCUSSION

Concealed damage has been found in peanuts grown on all types of soil, following various methods of fertilization and cropping practices and in peanuts cured in windrows, cocks, and stacks of different sizes and shapes. Sometimes there is less than 2 per cent damage in stacked or cocked peanuts, the hay of which is almost completely rotted. In other instances, there may be 5 to 10 per cent damage in peanuts having bright green, well cured hay.

The findings reported above help to clarify the questions regarding the cause of concealed damage. The figures show that the total length of time required for peanuts to cure is of less importance than the length of time required to complete the curing process after it has started. Peanuts that are stacked green in the rain and subjected to damp rainy weather for a week or more retain their moisture content at a high level. However, there is no appreciable development of damage until curing starts. If the curing process is rapid after it starts, the peanuts will contain relatively little damage. If, on the other hand, curing starts and the moisture content drops to approximately 30 per cent and the rainy weather sets in for several days the peanuts are likely to contain considerable concealed damage even though the hay is bright and well cured.

Since the pathogenicity of *Diplodia theobromae* has not been established by inoculation and reisolation,[4] the conclusion that this fungus causes the disease is open to question. However, this fungus makes up almost 90 per cent of all cultures obtained from peanut seeds having concealed damage;

[4] After this manuscript was accepted for publication Garren and Higgins (Phytopath. 37: 512–522. 1947) reported the production of concealed damage in surface disinfected peanut seeds by inoculation with either *Diplodia sp.* or *Sclerotium batatioola*.

and, in the early stages of the disease, it is the only fungus that can be isolated by the techniques employed in these investigations. These facts have been demonstrated over a period of 3 years, during which time over 2,500 seeds from more than 20 different samples have been plated. All of the evidence is in favor of *D. theobromae* being the principal causal agent of concealed damage in southeastern Alabama.

Since *Diplodia theobromae* is universally present in the peanut fields of southern Alabama there is little, if anything, that can be done to prevent infection. The extent to which these infections develop can be regulated. By following practices that promote rapid curing the grower nearly always can keep the percentage of concealed damage to less than the 2 per cent that is allowed without penalty.

Although stacking peanuts is a laborious and an expensive process, it is the only dependable method that is known at the present time by which the grower can produce a quality product. Dixie Runner, or other resistant varieties probably will replace those being grown at present, thereby reducing the importance of concealed damage. There is also the possibility that mechanized methods of harvesting will be developed that will eliminate weather hazards and thus help to standardize the quality of the peanuts offered for sale.

SUMMARY

1. Concealed damage of peanuts is a type of seed decay that begins on the inside of the seed and is not visible until the seed is broken open.

2. Damaged seeds have a strong rancid taste that renders them unfit for the confectionery trade. This rancidity is associated with an increase in the percentage of free fatty acid.

3. Most, if not all, of the infections occur in the field before the peanuts are dug. The extent to which the infection develops depends upon the way the peanuts are handled after digging.

4. The rate of curing is the most important factor affecting the development of concealed damage.

5. Concealed damage develops fastest in peanuts containing 15 to 35 per cent moisture. Above and below this moisture level the development is slower. If the peanuts have 10 per cent or less moisture at the time they are stored, the disease makes no appreciable progress.

6. The development of concealed damage is not associated with any particular soil type or fertilizer practice. The disease appears to be more common on land that is cropped to peanuts continually.

7. The fungus most frequently isolated from damaged seeds is *Diplodia theobromae*, one of the imperfect stages of *Physalospora rhodina*. Other fungi, species of *Fusarium* and *Penicillium*, *Sclerotium bataticola*, and other soil inhabiting fungi, are sometimes isolated but in most instances these fungi seem to come in as secondary invaders after the seed reaches the advanced stage of decay.

8. In the field the disease is most common in peanuts cured in cocks. Windrow-cured peanuts, as a rule, contain less concealed damage than stacked or cocked peanuts.

9. The disease usually is unimportant in Spanish peanuts.

10. A new variety of runner peanuts, Dixie Runner, appears to be rather resistant to the disease.

ALABAMA AGRICULTURAL EXPERIMENT STATION,
    AUBURN, ALABAMA.

LITERATURE CITED

1. CARVER, W. A., and FRED H. HULL. Peanut Improvement. Ann. Rept. Florida Agric. Exp. Sta. p. 37. 1945.
2. HIGGINS, B. B. ''Concealed Damage'' of runner peanuts. Georgia Agric. Exp. Sta. Press Bul. 536. 1944.
3. STANSBURY, MACK F., JOHN D. GUTHRIE, and T. H. HOPPER. Analyses of peanut kernels with relation to U. S. Standards for farmers' stock peanuts. Oil and Soap 21: 239–247. 1944.
4. VOORHEES, R. K. Life history and taxonomy of the fungus *Physalospora rhodina*. Florida Agric. Exp. Sta. Tech. Bul. 371. 1942.
5. WILSON, COYT. A Diplodia associated with concealed damage in peanuts. Phytopath. 35: 480. 1945.
6. —————, and J. P. WILSON. Field studies on the relationship between the method of curing and the development of concealed damage in runner peanuts. U. S. Dept. Agr., Pl. Dis. Reptr. 29: 61–63. 1945.
7. WOODRUFF, J. G., and S. R. CECIL. Studies with peanuts and peanut products. Georgia Agric. Exp. Sta. Publ. (Not numbered and not dated.)

BLUE-BLACK DISCOLORATION OF SPANISH PEANUTS[1]

KENNETH H. GARREN, B. B. HIGGINS, AND
J. G. FUTRAL[2]

INTRODUCTION

Spanish peanuts marketed by Georgia farmers are frequently graded down because of a prominent blue-black discoloration of a large percentage of the seeds. These discolored seeds often work through trade channels to confectioners and other processors. Development of this discoloration sufficient to be troublesome does not occur every season. In some years buyers and shellers find such a small percentage of blue-black discolored seeds that they do not report discoloration as a reason for grading down peanuts. There are seasons, however, during which peanut shellers have reported losses up to twenty-five per cent resulting from this discoloration. In such seasons entire lots are often rejected by peanut brokers.

In the southwestern United States this discoloration is known as "blue-damage."[3] The condition, however, has not been given a definite name in other peanut producing sections. The discoloration undoubtedly is frequent on Spanish peanut seeds wherever this variety is grown, but it has never been reported on other types of peanuts. Since Georgia is the center of Spanish peanut production, the discoloration has become of more concern here than elsewhere.

Because of its economic importance, an investigation of this discoloration has been undertaken. Out of this study have come definite conclusions concerning the cause of the discoloration, an explanation of its sporadic appearance, and suggestions for its control.

DESCRIPTION

As a general rule, when this discoloration is found in a seed lot, a large proportion of the seeds are conspicuously affected. Occasionally, however, lots have been found in which only a very few seeds were conspicuously discolored. Examination of samples from many fields throughout Georgia has shown that the discoloration may occur in such an inconspicuous form and on so few seeds that it may be easily overlooked. There is, then, a wide variation between lots both in percentage of discolored seeds and in intensity of the discoloration.

As it appears on the seed coat this discoloration varies through several shades of blue-black. One discolored spot may be of several shades, or dif-
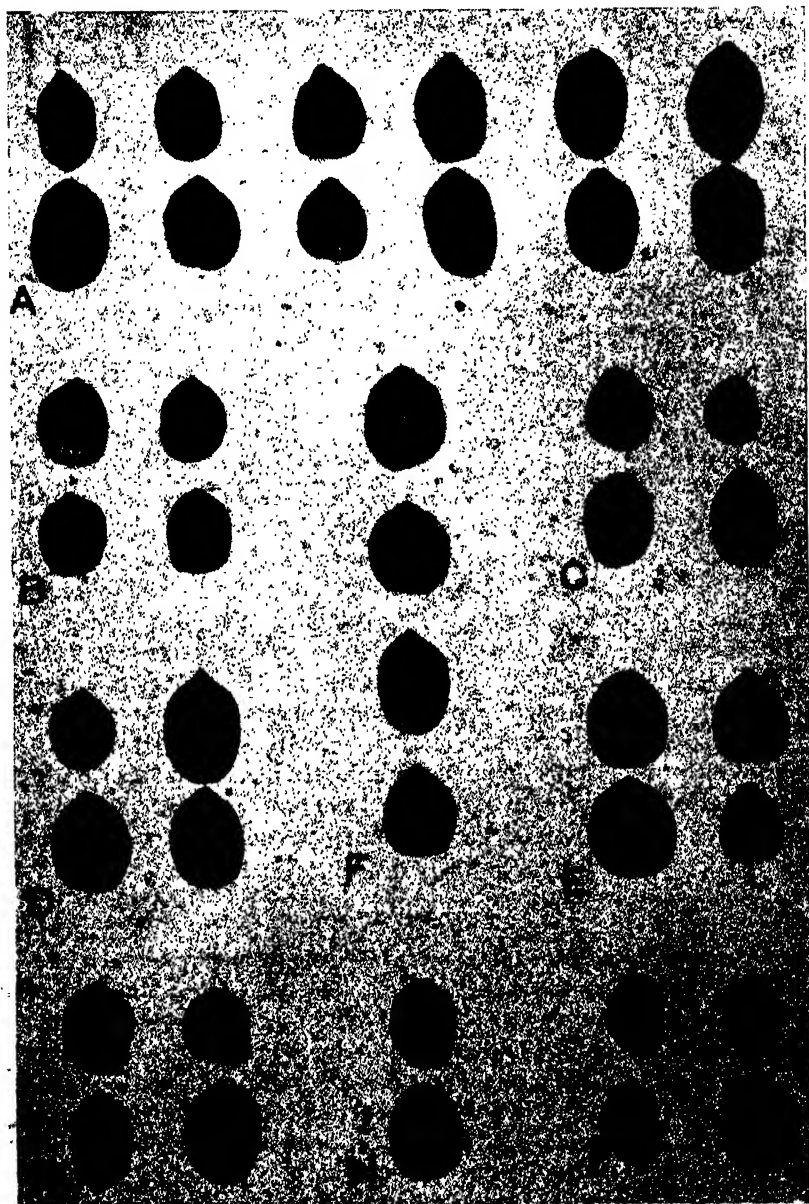
FIG. 1. Blue-black discoloration of Spanish peanut. A. Variation in size and shape of discolored areas on seed coats of peanuts cured in stacks. B. Discolored peanuts from farmer's stock in Texas, 1946. C. Discoloration produced by inoculating cured peanuts with sclerotia of *Sclerotium rolfsii*. D. Discoloration produced by placing crystal of oxalic acid against pods on living plants. E. Discoloration produced by soaking green peanuts in expressed juice of autoclaved culture of *Sclerotium rolfsii*. F. Nondiscolored peanuts from farmer's stock in Georgia, 1946. G. Seeds from farmer's stock peanuts with seed coats removed, showing discoloration of cotyledons associated with blue-black discoloration of the seed coats. H. Nondiscolored seeds from farmer's stock peanuts with seed coats removed. I. Discoloration of cotyledons associated with seed coat discoloration produced artificially with oxalic acid.

ferent spots on the same seed may be of different shades. Usually the discoloration is limited to definite spots (Fig. 1, A). In a few instances, however, the discoloration may appear as a streak following veins of the seed coat or the suture between the cotyledons. The spots vary greatly in size, the smallest being about two mm. in diameter. Small spots are circular, with a center which may be bleached, slightly darker than the seed coat, or the natural color of the seed coat. This gives a distinct "bull's-eye" effect. Large spots are irregular in shape, with no evident center; sometimes they appear to result from coalescence or overlapping of smaller spots. Although discoloration is most frequently found surrounding the hilum, it is not confined to this area. All possible variations in discoloration of individual seeds can be found in a single lot of seeds discarded by peanut processors.

When the seed coats are removed from blue-black discolored seeds, it is found that a considerable proportion of the cotyledons are not discolored, and that an even greater proportion have either a yellow or blue-black discoloration which is so faint as to be discernible only upon close observation.

TABLE 1.—*Relationship of external shell characteristics to blue-black discoloration of Spanish peanut seeds*

Appearance of shell	Total number of seeds	Percentage of discolored seeds
With evident mat of fungus hyphae	122	36.36
Prominent black discoloration and/or evident lesions	193	33.33
Faint black discoloration	126	22.22
Black discoloration or lesion at point of peg attachment	196	21.87
Normal	305	6.23

For example, of one lot of 250 seeds with blue-black discolored seed coats, about one-third (37 per cent) had no detectable discoloration of the cotyledons, about one-half (52 per cent) had cotyledons faintly discolored, and about one-tenth (11 per cent) had cotyledons conspicuously discolored. This cotyledon discoloration is illustrated in figure 1, G.

Certain external shell characteristics are correlated with the presence of blue-black discolored seeds (Table 1). Field lots containing discolored seeds generally have more discolored or otherwise damaged shells than do lots which do not contain discolored seeds. Data in table 1 were obtained by a random sampling of field stacks. When shells which had contained discolored seeds were placed in moist chambers, several fungi grew out of the shells. The predominant organism was *Sclerotium rolfsii* Sacc. There was some growth of *S. rolfsii* from shells which had not contained discolored seeds, but not to the extent that this organism developed from the shells which had contained discolored seeds.

In table 2 the nitrogen and oil content of discolored seeds, including seed coats, is compared with the chemical composition of nondiscolored seeds from the same curing treatment and with the chemical composition of nondis-

colored seeds from other curing treatments.[4] From these data it is evident that the discoloration has not resulted in major changes in important chemical constituents of the seeds. In a test for rancidity, discolored seeds from a single curing stack were peeled and checked against peeled nondiscolored seeds from the same stack. These seeds were checked by eleven people, none of whom could detect any odor of rancidity in either seed group. Even though the discolored cotyledons in one seed group made it appear that there might be an off-flavor, six of the eleven people could not detect any off-taste in the discolored seeds. The remaining five thought that the discolored seeds had a slightly bitter taste. These peeled seeds were roasted at 325° F. for 25 min. and ground until there was no difference in appearance of the two lots. These roasted lots, labeled "A" and "B," were tasted by

TABLE 2.—*Nitrogen and oil content of blue-black discolored and non-discolored Spanish peanut seeds*

Sample No.	Curing treatment	Types of seed	Composition, air-dry basis		
			Moisture	Nitrogen	Crude oil
			Per cent	*Per cent*	*Per cent*
1	Stack	Discolored	4.76	4.12	48.06
2	Do	Nondiscolored seeds from 1	4.71	4.08	47.84
3	Do	Discolored	5.14	4.38	49.15
4	Do	Nondiscolored seeds from 3	4.79	4.28	47.07
5	Windrow	Nondiscolored	4.81	4.02	47.12
6	Picked off green	Nondiscolored	5.05	3.90	48.33
7	Stubs from topped plants	Nondiscolored	4.52	4.10	48.30

24 people. There was no agreement in the reaction of the tasters. The ground nondiscolored seeds were picked for off-flavor as often as were the ground discolored seeds, and 10 of the 24 tasters felt that there was no difference in taste between the two ground lots.

Approximately 6,000 discolored seeds and the same number of nondiscolored seeds were germinated in field soil. No difference was noted between the percentage emergence of plants from the discolored seeds as compared with the nondiscolored seeds. The plants from discolored seeds appeared fully as vigorous as those from nondiscolored seeds.

ETIOLOGY

Numerous attempts to isolate fungi from the discolored spots have been made. After surface sterilization, however, no fungus grew from the great majority of the discolored seeds. When a fungus was isolated, it was usually *Sclerotium rolfsii;* but this organism grew from less than one per cent of the seeds plated. It seemed apparent, therefore, that the discoloration is the result of a chemical reaction between some extraneous material and the

[4] Chemical determinations by Mr. T. A. Pickett, Department of Chemistry, Georgia Experiment Station.

pigments of the seed coat. The porous peanut shell will act much as a sponge in absorbing materials which can then gradually seep into the seed coat.

In considering possible sources of extraneous materials it was noted that: (1) *Sclerotium rolfsii* is prevalent in most of the fields in which Spanish peanuts are grown, (2) *S. rolfsii* was isolated from a few of the discolored spots, (3) *S. rolfsii* grew readily from shells of peanuts containing blue-black discolored seeds, and (4) it has been shown that the hyphae of *S. rolfsii* secrete oxalic acid and that this acid diffuses into plant tissue in advance of any penetration of the hyphae.[5] A number of experiments were designed to test the hypothesis that the discoloration results from a reaction of oxalic

TABLE 3.—*Laboratory production of blue-black discoloration of Spanish peanut seeds*

Treatment No.	Description of treatment	Total number of seeds	Percentage of discolored seeds	Intensity of discoloration
1	Green peanuts soaked 24 hours in drippings from autoclaved culture of *Sclerotium rolfsii*	132	63.6	Very faint
2	Green peanuts soaked 48 hours in expressed juice of auto-claved culture of *Sclerotium rolfsii*	184	75.0	Medium intense
3	Same as treatment No. 2, but partially cured peanuts were used	147	40.4	Medium intense
4	Crystal of oxalic acid kept against nuts on living plants in damp soil for 72 hours	319	66.5	Intense
5	Well cured peanuts inoculated in moist chamber with sclerotia of *Sclerotium rolfsii*	826	29.3	Very intense
	Control, nuts from same plants as those used in treatments 1, 2, 3, and 5	350	0.0	....

acid, secreted by *S. rolfsii*, on the seed-coat pigments.   Table 3 gives the methods and results of the most significant of these experiments.

It was concluded that oxalic acid secreted by the hyphae of *Sclerotium rolfsii* reacts on pigments of the seed coat of Spanish peanuts to produce a typical blue-black discoloration. Inoculation, with *S. rolfsii*, of peanuts known to contain no blue-black discolored seeds resulted in the production of a considerable percentage of blue-black discolored seeds (Table 3, Treatment 5; and Figure 1, C). The discolored areas on these seeds are darker than the discolored areas on seeds from the field. This darker shade is probably due to a chemical change in the pigments of the seed coat during curing. The oxalic acid that seeped through the shells of fresh nuts in damp soil (Table 3, Treatment 4) produced a seed-coat discoloration which was almost identical with that found in discolored lots from the field (Figure 1, D).

[5] Higgins, B. B.   Physiology and parasitism of *Sclerotium rolfsii* Sacc.   Phytopath. 17: 417–488.   1927.

The resulting discoloration of the cotyledons was also comparable to that found in field lots (Figure 1, I).   Application of liquid from an autoclaved culture of *S. rolfsii* to green and partially cured nuts (Table 3, Treatment 1, 2, and 3) resulted in a lighter form of the discoloration in which the interior of the discolored area was noticeably bleached.   The liquid contained approximately 1.7 per cent oxalic acid.[6]

Discolored seed coats and cotyledons from both field lots and lots in which the discoloration was produced artificially were subjected to qualitative tests for oxalic acid and oxalates.[6]   Several tests were used, following various standard procedures.   There was no indication of oxalic acid or oxalates in either of the two types of discolored seeds.   No crystals of cal-

TABLE 4.—*Results of field studies on relation of curing method and weather during curing to development of blue-black discoloration of Spanish peanut seeds*

Lot No.	No. of samples in lot	Curing method	Weather during curing	Total number of seeds observed	Percentage of discolored seeds	Intensity of discoloration
1[a]	28	Wilted, dry plants stacked in field	Hot and dry	5,000 +	0.00	....
2	5	Do	Do	2,000 +	0.78	Very faint
3	3	Do	Do	7.12	2.24	Do
4[b]	3[c]	Green, damp plants stacked under shed	Warm and damp	599	70.54	Very intense
5	3	Green, damp plants stacked in field	Do	542	16.67	Do
6	3	Green, damp plants windrowed under shed	Do	462	0.55	Very faint
7	8	Plants topped. Nuts and roots spread under shed	Do	466	0.00	....
8	3	Green nuts picked, spread under shed	Do	491	0.00	....

[a] Lots 1 through 3 from fields in Crisp County, Georgia; harvested during September, 1946.

[b] Lots 4 through 8 from field in Spalding County, Georgia; harvested late October, 1946.

[c] Prominent mats of *Sclerotium rolfsii* noted throughout stacks when samples for lots 4 and 5 were taken.

cium oxalate or similar compounds could be found by microscopic examination of macerated discolored seed coats and cotyledons.

Development of blue-black discoloration in the field was studied in Georgia in the fall of 1946.   Most of the Spanish peanuts grown in Georgia that year were harvested and cured during a period of hot, dry weather. Although many of these peanuts were harvested from fields heavily infested with *Sclerotium rolfsii*, early checks made on samples from several fields showed that there had been no noticeable development of the discoloration in the main crop.   Thirty-six samples from the main Spanish peanut producing area of Georgia were examined carefully.   None of these samples

[6] Chemical analyses by Dr. L. C. Olson, Georgia Experiment Station.

contained any seeds conspicuously discolored, and only eight samples contained seeds faintly discolored (Table 4, Lots 1, 2, and 3).

In the middle of December inquiries were made of buyers and shellers, and no one reported any noticeable percentages of discolored seeds in peanuts marketed up to that time. In early February two of the large peanut brokerage houses which had previously reported no discolored seeds were visited, and their Spanish peanuts being prepared for shipment were examined. Spot checks showed some lots of peanuts with about one per cent of the seeds prominently discolored. There was no way of determining when the lots containing these discolored seeds had been placed in the storage bins. There are, however, three main possibilities: (1) The discolored seeds could have come from lots purchased before the middle of December, but overlooked in the broker's previous report. (2) The discolored seeds could have come from lots purchased after the middle of December, and, therefore, harvested and cured later than the main crop of 1946. (3) The discoloration could have developed after the nuts had been placed in storage. In connection with the last possibility, one buyer stated that he had placed in bins many lots of peanuts he knew to be too damp for proper storage.

In continuing the field study on development of the discoloration, a late planting of Spanish peanuts at Experiment, Georgia, was selected. This planting was in a field severely infested with *Sclerotium rolfsii*, and the crop was harvested and cured during a period of warm, damp weather. The plants were dug and then cured by five different methods. After curing, the percentages of blue-black discolored seeds were determined for each curing treatment. The methods and results of this experimental work are given in table 4, Lots 4 through 8.

An inter-relationship exists between curing methods and weather conditions and development of the discoloration (Table 4). The absence of prominent discoloration in peanuts cured in stacks during hot, dry weather and the pronounced development of discoloration in peanuts cured in stacks during warm, damp weather emphasize the importance of weather conditions. Weather conditions determine rapidity of drying of the curing plants and thus influence the amount of discoloration which develops.

Moisture retained in the curing plants affects the development of blue-black discoloration (Table 4). Curing under a shed kept the sunlight from the plants and decreased circulation of air through the stack. Almost five times as much discoloration developed in the stacks under the shed as in the stacks in the field. This may be attributed to the difference in rapidity of drying of the stacked plants in the two localities. When peanuts from this same field were cured under conditions that promoted rapid drying, conspicuously discolored seeds were completely lacking (Lots 6, 7, and 8); and only one curing treatment, windrowing under shed, resulted in faintly discolored seeds (Lot 6).

Since the blue-black discoloration did not develop in some lots (Lots 7 and 8) of peanuts taken from the same field as those lots (Lots 4 and 5) in

which it did develop, it is apparent, in this case, that the discoloration developed after the peanuts had been removed from the soil. If *Sclerotium rolfsii* is associated with this discoloration, then the growth of *S. rolfsii* which resulted in this discoloration took place after the plants had been removed from the soil. Plants for all but Lot 2 of the eight lots in table 4 were examined at digging time and the majority of the plants which went into each of these seven lots showed indications of the presence of *S. rolfsii*. When threshed, only the plants of Lots 4 and 5 showed any indication that there had been a continued development of *S. rolfsii*. In these lots mats of the fungus were evident throughout the entire group of stacks. Thus, *S. rolfsii* had grown from infested tissue of some of the curing plants, and had spread, saprophytically, throughout the mass of curing peanuts. The presence of conspicuous blue-black discoloration only in these lots which showed abundant development of *S. rolfsii* in the curing stack is taken as proof that the discoloration developed in the field as a result of the action of oxalic acid secreted by *S. rolfsii* as it grew over the curing peanuts.

DISCUSSION

Blue-black discoloration of Spanish peanuts makes the seed unsightly. This, in itself, is cause for concern since it is considered sufficient reason for grading down peanuts. Processors would hesitate to use such seeds in the manufacture of food products, even if food production regulations did not prohibit their use. Some of the results reported indicate a possibility that these discolored seeds are not sufficiently different from nondiscolored seeds to merit their rejection. It will be necessary to repeat many times these tests on taste, chemical composition, and germination of discolored seeds as compared with nondiscolored seeds. Only then will it be safe to recommend either the use or rejection of these discolored seeds.

It is possible to eliminate a large proportion of discolored seeds before sending the peanuts to the shelling machines, but certain precautions should be noted. Damaged shells of the types listed in table 1 are frequently found in lots of Spanish peanuts which do not contain discolored seeds. This means that it is first necessary to determine, by preliminary sampling, that the discoloration is found on the seeds of a given lot of peanuts before the shell characteristics may be used as criteria for judging the presence of discolored seeds.

A seed discoloration typical of that found in field lots was produced by an extraction of *Sclerotium rolfsii* seeping through peanut shells, and also by inoculation of peanuts with *S. rolfsii*. Therefore, some substance secreted by *S. rolfsii* may be instrumental in producing the discoloration. Since it is known from previous work[7] and from determinations on culture extracts that *S. rolfsii* secretes oxalic acid, and since oxalic acid seeping through the shells of peanuts also produced the discoloration, the substance involved appears to be oxalic acid. The fact that *S. rolfsii* was isolated from very

[7] See footnote 8.

few of the discolored seeds plated indicates that sufficient oxalic acid to produce the discoloration is secreted by the fungus growing saprophytically in or on peanut shells. The absence of detectable amounts of oxalic acid or oxalates in discolored seeds suggests that the discoloration results from an indicator reaction, and that the oxalic acid is decomposed in the reaction.

The evidence connecting oxalic acid secreted by *Sclerotium rolfsii* with the discoloration does not eliminate the possibility that the discoloration may sometimes result from the action of organic acids secreted by other fungi. *S. rolfsii*, however, is prevalent in most peanut fields, and is frequently found growing rapidly and abundantly over curing peanut plants. It is highly improbable, therefore, that other fungi are so important as *S. rolfsii* in producing this discoloration.

It may be assumed that this discoloration develops, for the most part, during the curing process. As shown by lots in which only a few faintly discolored seeds are found, the development may be arrested at a very early stage. In these cases it is possible that the growth of *Sclerotium rolfsii* which resulted in the discoloration began while the peanuts were still in the soil and was stopped by removal of the peanuts from the soil and their subsequent rapid drying. Then, too, there is a darkening of the pigments of the seed coat of peanuts during the curing process. Perhaps this discoloration develops most readily when the acid reacts with the pigments at the same time that the pigments are undergoing the chemical change which makes them darker. Sometimes peanuts left in the soil until they have become over-mature may have discolored seeds when dug. It is considered probable, also, that some discoloration may develop during storage if peanuts are stored with sufficient moisture in the shells to promote the growth of *S. rolfsii* or other acid-secreting fungi which may be present in or on the shells. In general, however, the discoloration which may develop at times other than during the curing process may be considered as unimportant.

It is also evident that as far as stacking is concerned, weather conditions during curing will have considerable influence on development of the discoloration. Since most Spanish peanuts grown in Georgia are cured in stacks, this effect of weather is also an explanation for the sporadic appearance of the discoloration. Little or no discoloration results when stacked peanuts are cured during hot, dry weather. Thus, when this type of weather holds during the curing season, the stacked plants will dry out rapidly and will not retain enough moisture to support the growth of *Sclerotium rolfsii* in the stacks. Conversely, considerable discoloration of seeds results when stacked peanuts are cured during warm, damp weather. When this type of weather obtains, during the curing season, the stacked plants will not dry out readily and there will be ample opportunity for continued growth of *S. rolfsii* from infested plants in the stack. The situation in regard to stacked peanuts in general is somewhat complicated by treatment of the harvested plants before stacking as well as by weather changes which take place after stacking. Wilting the plants before stacking makes a looser

stack and speeds the drying process, whereas stacking green plants makes a compact stack in which drying is slow. Also, if stacked peanuts which have dried out are subjected to wetting during curing, any *S. rolfsii* present may be able to grow before the plants become dry again.

Considerable development of this discoloration is reported from Texas where field-windrowing is the generally used method of curing peanuts. Field windrowing, therefore, is not the answer to the problem of controlling this discoloration. Absorption of moisture from the soil and from dew apparently maintains sufficient moisture in curing, field-windrowed plants to promote growth of *S. rolfsii*. Then too, the majority of field-windrowed plants will be in direct contact with the soil so that there will be ample opportunity for *S. rolfsii* to grow directly from the soil and spread through the curing plants.

Sclerotium rolfsii is prevalent in most Spanish peanut fields. This means that, since weather conditions cannot be controlled, and since field-windrowing does not prevent development of this discoloration, the Spanish peanut grower has three choices remaining: First, he may stack his peanut plants green and hope that weather conditions will promote a rapid drying out of the stacked plants. Second, he may cure his peanuts by some modification of rapid drying methods used in this study. Third, he may wilt the peanut plants before stacking them, then stack them carefully with the nuts inside. If the interior of the stacks become wet later, a loosening-up of the stacks may speed the redrying. Because of limited space, control of the discoloration by the second method cannot be practiced generally. The third method is subject to limitations in that the stacks may sometimes become wet and not redry sufficiently soon to prevent growth of *S. rolfsii*. This method, however, is the best means known at present for controlling the discoloration.

SUMMARY

A blue-black discoloration of seeds which appears sporadically in Georgia is a frequent cause for grading down Spanish peanuts. There is considerable variation between seed lots in percentage of seeds discolored and in intensity of the discoloration. Usually, however, when the discoloration occurs, a considerable proportion of the seeds are conspicuously discolored.

Some external shell characteristics are associated with the presence of blue-black discolored seeds. These characteristics, however, are sometimes found on shells that do not contain discolored seeds.

Evidence, which is preliminary in nature, indicates that these blue-black discolored seeds are not noticeably different in flavor, chemical composition, or germination from seeds not discolored.

It is concluded that the discoloration is the result of an indicator reaction involving the pigments of the seed coat and oxalic acid secreted by *Sclerotium rolfsii*. Other fungi which secrete organic acids may sometimes be involved, but the prevalence of *S. rolfsii* in peanut fields indicates that it is by far the most important organism associated with the discoloration.

Field studies indicate that, in general, the discoloration develops as a result of the saprophytic growth of *Sclerotium rolfsii* over peanuts during curing. Development of the discoloration in peanuts still in the soil and in stored peanuts is regarded as negligible. When Spanish peanuts are cured under weather conditions which promote the retention of considerable moisture in the curing plants, the discoloration is found on a large percentage of the cured seeds. Some curing methods also promote this retention of moisture in curing plants. The sporadic occurrence of these weather conditions, rather than the curing method, is regarded as an explanation for the sporadic appearance of the discoloration.

Weather conditions or curing methods which result in rapid drying-out of the curing peanuts and the maintenance of this dry condition result in few or no discolored seeds. Control methods, therefore, will have to be centered in the curing procedure.

The most practical method of inhibiting or preventing the development of this discoloration is to stack wilted plants carefully and attempt to speed the drying of the stacks if they become wet during the curing process.

DEPARTMENTS OF BOTANY AND AGRONOMY,
  GEORGIA AGRICULTURAL EXPERIMENT STATION,
    EXPERIMENT, GEORGIA.

Notes on Physiologic Specialization in Leaf Rust of Wheat in China.[1]—
Leaf rust is one of the most important diseases of wheat, especially in the
regions along Yangtze River in China. Information on the number, dis-
tribution, and prevalence of the physiologic races of *Puccinia rubigo-vera
tritici* is urgently needed as an invaluable aid in the production of rust-
resistant varieties.

Investigations were started in 1940. Rust collections were made from
various places in Yunnan, or sent in from Kwangsi, Kweichow, and Szechwan
provinces in the southwestern part of China. Isolations were made from a
single uredium in each collection, and the rust cultures from each uredium
almost always appeared to be pure. Eighty-nine single uredium isolates
were studied during 1940–1942. Only 3 races, namely, races 1, 63, and 123,
have been identified in the collections made both in 1940 and 1941.

Among the 8 differential varieties, only Brevit varied greatly in its reac-
tions toward the different races studied (Table 1). All other varieties ap-

TABLE 1.—*Reactions of differential varieties of wheat to physiologic races of
Puccinia rubigo-vera tritici collected in the southwestern provinces of China in 1940 and
1941*

Rust race	Infection type on							
	Malakof	Carina	Brevit	Webster	Loros	Mediter-ranean	Hussar	Democrat
1	0	0–1	0–2	0	0–1	0–1	0	0
63	0	0–2	2–3	0–2	0–1	0	0	0
123	0	0–2	3–4	0–2	0–1	0	0	0

peared to be resistant to the three races. The reactions of the differential
varieties to race 123 appeared to differ from reactions to any of the 108 races
registered up to 1939.[2] This race was temporarily designated by the writer
as a new race in 1940, and it was designated race 123 only after Asuyama's[3]
work was reported.

The 3 races occurred rather generally in the southwestern provinces. No
definite geographical distribution can be made until more collections are
studied. Race 123 is apparently the most prevalent race, constituting more
than 55 per cent of the collections studied (Table 2). Races 1 and 63 fol-
low, their prevalences being almost equal.—H. R. WANG, Department of
Plant Pathology, Peking University, Peiping, China.

TABLE 2.—*Number, distribution, and prevalence of the physiologic races of Puccinia rubigo-vera tritici in southwestern provinces of China in 1940 and 1941*

Sources of collection	No. of collections of			Total no. of collections
	Race 1	Race 63	Race 123	
Yunnan Province				
Kunming	1	5	22	28
Cheng-kung	2	1	1	4
Iliang	1			1
Kou kai	2		2	4
Lunan	1	1	4	6
Pansi	3	2	3	8
Yusi	1			1
Tunghai		1	1	2
Kaiyuan		1	1	2
Kweichow Province				
Tsing-lung	1			1
Kweiyang	2	2	5	9
Meitan	5		5	10
Kwangsi Province				
Liuchow		1	1	2
Kueiling			1	1
Szechwan Province				
Yung chang	2		1	3
Chengtu	1	4	2	7
Totals	22	18	49	89
Percentage prevalence	24.7	20.2	55.1	

Brown Stem Rot of Soybean Caused by a Cephalosporium.[1]—A serious disease of soybean has been previously reported and the causal organism isolated and its pathogenicity proven.[2]   The identification of this fungus, however, has been heretofore impossible because no fruiting on the host or laboratory culture media has been observed.   This note describes methods for inducing sporulation by this fungus on culture media, and the basis for classifying it with the genus *Cephalosporium*.

Cultures of the brown-stem-rot fungus produced conidia sparsely on potato-dextrose agar, oatmeal agar, and potato-dextrose-raisin agar; however, no conidiophores with conidia attached were observed.   Growth of the fungus on these media was extremely slow.   It was found that the spores could be suspended readily in sterile water by gently flooding the surface of a two-month-old culture.   These spores, when planted on 2 per cent water agar, germinated readily, and conidiophores and conidia were produced abundantly in 8–10 days at room temperature.   Younger cultures were induced to fruit by dividing a small portion of the mycelial mat very finely and placing the fragments on water agar.   Two other media have been found satisfactory for inducing sporulation of this fungus.   Dr. B. W. Henry, Camp Detrick, Maryland, who was given a culture of this organism by the junior author for sporulation studies, found that 2 per cent rice polish

[2] Allington, William B.   Brown stem rot of soybean caused by an unidentified fungus. Phytopath. 36: 894.   1946.

added to 2 per cent agar was a good substrate for sporulation of the fungus. Perhaps the best sporulation has been found on a medium consisting of 2 per cent agar to which was added an extract of green soybean leaves and stems before autoclaving. Fifteen to twenty grams of green plant tissue were pulverized in a Waring Blendor in 100 cc. water. This mixture was then filtered through cheesecloth and added to 900 cc. of the agar-water preparation. Growth on this medium was less dense and more prostrate than on the potato-dextrose agar. Addition of sucrose to the medium greatly inhibited or eliminated sporulation.

The conidia are produced in irregularly shaped heads and disperse instantaneously on contact with water. The conidia are ellipsoidal, hyaline, and very small (approximately $1.5\text{–}2.5 \times 2.0\text{–}5.0\ \mu$). The conidiophores are short and usually unbranched.

A culture was examined by John A. Stevenson, Principal Mycologist in Charge, Mycology and Disease Survey, U. S. Department of Agriculture, who substantiated the conclusion that the fungus was a *Cephalosporium*. Dr. B. W. Henry independently identified the organism as a *Cephalosporium*. Stevenson kindly furnished a culture of *C. acremonium* Cord. for comparison with the brown-stem-rot organism. In preliminary tests the brown-stem-rot organism was not parasitic on corn as *C. acremonium* is reported to be. The soybean fungus grows much more slowly on all culture media studied and is less prone to fruiting. It is likely that further studies now in progress will establish this parasite as a new species of *Cephalosporium*.—JOHN T. PRESLEY, Division of Cotton and Other Fiber Crops and Diseases, U. S. Department of Agriculture, and Mississippi Agricultural Experiment Station, State College, Mississippi, and WILLIAM B. ALLINGTON, Division of Forage Crops and Diseases, U. S. Department of Agriculture, Urbana, Illinois.

A Corrective Measure for "Soil Sickness" Occurring in Sand Media.[1]— "Soil sickness" or Bodenmüdigkeit has been recognized over a considerable period of time. The detrimental effects on plants by causes other than cultural practices, fertilizer, fungi, bacteria (and viruses) have been described under the term. Crops affected by "soil sickness" show a gradual decline in productivity which is often followed by death of the plants, and the inability of the same crop to grow on the same soil. Such phenomena may be observed readily in certain regions of Germany where crops such as grapes, cherries, and plums have been planted for a long time. Perhaps the die-back of peaches in North Carolina and the spreading-decline of citrus in Florida are due to a similar phenomenon, that is, "sand sickness."

A similar phenomenon may be observed frequently with the extended use of growing and propagation media in the greenhouse. When roses are grown over a period of time in sand or gravel culture, the plants eventually

grow poorly, the roots die, and the flower production declines. When chrysanthemum cuttings are rooted in the same sand over a period of two to three years, plants in certain sections of the bench become stunted, grow little or not at all, and in some cases they die. At irregular intervals the roots darken completely and start to distintegrate. Very frequently rust-colored lesions appear. These resemble necrotic lesions caused by soil-borne pathogens. Often no roots develop, and in many instances if they do, they are short, thickened, and gnarled. The plant fails to produce new roots, for as soon as they are formed, they darken and die.

Numerous attempts to isolate possible causal organisms from the lesions have met with no success. Bacteria and fungi isolated from the surrounding medium did not prove pathogenic if the plants were inoculated with them. No symptoms were observed on any of the plant parts inoculated. Soil sterilization with heat, formaldehyde, or chloropicrin did not correct this sand condition. The symptoms appeared on the plants whether the sand was sterilized or not. Attempts to correct this condition of "sand sickness" with liberal amounts of water (300 gal. per 1 cu. ft. of sand) gave erratic results. There was partial improvement in some instances and none in others. The best means of correcting this condition was removal of "sick sand" and replacement with new sand. For large areas this entailed considerable expense.

Dilute sulfuric acid had a corrective effect on the sand. Under the conditions of the experiments, it was found that a one per cent concentration of sulfuric acid was satisfactory when applied at the rate of 5 gal. per 20 sq. ft. of bench space having a depth of 4 inches. After the treatment, the sand was flooded with water in order to counteract whatever residual effect the sulfuric acid might have on the crops to be planted.

In order to test the effectiveness of the method, chrysanthemum plants which had been grown previously on sick sand, and in which the symptoms of sand sickness appeared, were transferred to treated sand. On this medium, they recovered completely and formed healthy new roots. Plants grown in the treated sand and then removed to "sick" sand succumbed to the "sand sickness."

The application of sulfuric acid was so effective that it was not necessary to remove plants from sick sand. By placing asphalt-coated, tin collars around the plants, then treating the outside area with sulfuric acid as described, new healthy roots invaded the treated sand a short time after removal of the tin collars. When oats were used as test plants (Neubauer test) it was found that the "sick sand" stunted the growth of young seedlings without causing any visible root injury, while in the treated sand the growth of the seedlings appeared normal.

It is believed that instead of frequent changes of sand or gravel under greenhouse conditions, the application of this method will greatly reduce production costs.—E. O. MADER, Division of Plant Pathology and Botany, University Farm, St. Paul, Minnesota.

BOOK REVIEWS

List of Common British Plant Diseases. Compiled by the Plant Pathology Committee of the British Mycological Society. 61 pp. Cambridge University Press. 1944. 5s net.

Arranged alphabetically by hosts, the list is a convenient compilation of the more common plant diseases occurring in the British Isles. In addition the list provides carefully considered and selected common names for plant diseases which, it is hoped, will encourage uniformity of usage. In most cases the preferred common name is one in common use in the United States. In divergent instances the American name is also listed. A valuable feature is the listing and indexing of many foreign common names of plant diseases, including French, Italian, German, Dutch, Danish, Swedish, Spanish, and Russian terms. The nomenclature of fungi is generally in accord with common usage and synonyms are included when they contribute to clarity. The Committee has apparently given much consideration to nomenclatorial principles and avoidance of undesirable changes in scientific names. Pending consideration of the problem at the next International Botanical Congress the Committee wisely avoided recommending any one system of virus nomenclature.

The hosts are arranged alphabetically by common names; however, an index of Latin names of hosts and parasites makes the list usable by those unfamiliar with English common names. The careful attention accorded preferred common names, nomenclature and indexing gives the list unusual value.—C. M. TUCKER, University of Missouri, Columbia, Missouri.

GÄUMANN, ERNST O. *Pflanzliche Infektionslehre.* 611 pp., 311 figs. Verlag Birkhäuser. Basel, Switzerland. 1946. Swiss Fr. 48.50.

''*Pflanzliche Infektionslehre*'' is a thoroughly modern book on the general principles of plant pathology. It is well written in readable German, and is extremely valuable not only as a compendium of information on general principles of plant pathology but also as a reference book. It is not a mere description of plant diseases and prescriptions for their control; it is rather a book in which pathological phenomena have been grouped logically and in which principles are based on an abundance of scientific data.

The book is divided into six chapters, each subdivided in such a way as to make the table of contents a clear guide to the various materials included.

In Chapter 1 are discussed the phenomena of infection, including the methods of entrance of pathogens into host plants, the time required for infection, incubation, and fructification of the pathogen, the avenues of entrance, and the histological relationships between pathogen and host. The effect of environmental conditions and various other factors on the various processes are clearly and adequately discussed.

Chapter 2 is devoted to sources of inoculum, the various agents of dissemination, and the factors affecting the development of epidemics.

In Chapter 3 are considered the pathogenic potentialities of the various kinds of pathogens, including genetic differences and variability due to nuclear phases in the developmental cycle of the organisms, genetic and physiological changes in pathogens, the influence of the host under various conditions, and the interactions of various organisms in mixed infections.

Chapter 4 deals with disease resistance and susceptibility. The various factors affecting disease resistance are discussed, and the variability of resistance and susceptibility are well brought out. The development of resistant varieties and the mode of inheritance of disease resistance are discussed at some length. There is included also a good discussion of predisposition.

Chapter 5 is a good compendium of information on the nature of disease, including signs and symptoms and morphological and physiological changes resulting from disease.

Chapter 6, which is the shortest one in the book, gives the general principles on which methods of control are based. This includes prevention of infection, the use of resistant varieties, proper soil management, and chemotherapy.

Although the emphasis is on general principles, the book is useful practically also, because of the detailed discussions on the physiology, ecology, and genetics of plant pathogens in relation to development of disease. Likewise, the relations between the morphology, physiology, ecology, and genetics of host plants are used to explain the variable disease reactions of host plants.

It probably will be agreed by most plant pathologists that plant pathology is both an applied and pure science. Professor Gäumann's book is a good illustration of the fact that concentration on basic principles furnishes the soundest foundation for practical pro-

cedures. The complexity of the science, with its dependence on basic sciences, is clearly brought out by numerous, well-chosen examples. The many photographs, graphs, and tables add to the ease of comprehension of the facts and concepts in the book.

All plant pathologists will welcome this book, not only for its utility but also because of the fact that it shows how thoroughly scientific the study of disease phenomena in plants has become. It is refreshing and stimulating to read a book of this nature, in which examples to illustrate principles are chosen because of their pertinence rather than because of their economic importance. The book is a scholarly treatise for which pathologists owe Professor Gäumann a debt of deep gratitude.—E. C. STAKMAN, University Farm, St. Paul, Minnesota.

COOK, MELVILLE T. *Viruses and virus diseases of plants.* 244 pages, 20 illus. Burgess Publishing Co., Minneapolis, Minnesota (photo-offset). 1947. $4.00.

This book is an extensive review of the literature relating to plant viruses and should prove to be a handy and useful reference for both students and research workers on viruses. The book does not deal with specific diseases, or viruses as such, and in this respect would be less useful to the general plant pathologist or extension worker. Dr. Cook has divided the subject into six chapters as follows: (1) Introduction; (2) Theories as to Cause of Virus Diseases; (3) Nature and Properties of Plant Viruses; (4) Reaction of Hosts to Viruses; (5) Transmission of Viruses; and (6) Control of Virus Diseases of Plants. These chapters are, however, treated under a total of about 175 subtitles, listed in the table of contents. It is, therefore, relatively easy for the reader to locate a phase of the subject on which information is sought.

The text leans considerably toward the historical and chronological side of the subject. In this respect it is a valuable supplement to other textbooks dealing more intensively with recent experimental results, analyses, and interpretations of data. There is a tendency for new researchers in a field to overlook historical backgrounds that may often help to interpret data and develop new leads for investigation. Dr. Cook has recorded some of the earliest theories and conceptions as to the nature of virus diseases of plants. An especially interesting feature of the book is 12 chronological lists of selected contributions of knowledge to special phases of the subject, with the appendix including a list of about 300 "highlights" of the entire subject dating from 1576 to 1945. The bibliography contains about 1100 references, no doubt one of the largest yet published for plant viruses. An author index and general index is included.

The type of printing (photo-offset) no doubt accounts for only about 20 illustrations being used, and this detracts in some measure from the value of the book. The material on the whole is well organized, although one may disagree with certain groupings that seem to lead to some repetition and confusion. For example, Chapter 2 entitled "Theories as to Causes of Virus Diseases," includes as one of ten theories a subheading on "The Virus Theory." In a book covering the whole field of a subject as complicated as the plant viruses, even though no consideration is given to the animal viruses, it is not surprising that some important contributions are omitted and that other less significant or even disproved conclusions are accepted. The author resorts more frequently to direct quotations from original papers than may be justified by the circumstances. Although Dr. Cook has presented interpretations and short summaries in some sections of the book, there is more need for it in other sections with the purpose of starting the beginning student off in the safest direction. In view of Dr. Cook's long experience in the field of plant pathology, it is unfortunate that these discussions are not more critical. Inaccuracies and typographical errors are not unusual in number for a publication of this size. Altogether this book summarizes our knowledge of plant viruses in a convenient and readable form, and copies of it should be made available in biological libraries to all students of plant pathology. The virus specialist may find opportunity for disagreement and criticism, but it is on such soil that the science of the viruses appears to thrive.— JAMES JOHNSON, University of Wisconsin.

ANNOUNCEMENT

The thirty-ninth annual meeting of The American Phytopathological Society will be held with A.A.A.S. at the Hotel Stevens in Chicago, Illinois, December 28–31, 1947. There will be joint sessions with The Potato Association of America, The Botanical Society of America, and The Mycological Society of America. All meetings will be held in Hotel Stevens.

Abstracts of papers to be presented at the meeting must be in the Office of the Secretary of The American Phytopathological Society by October 15, 1947.

FRED CARLETON STEWART

1868–1946

HARRY M. FITZPATRICK[1]

Fred Carleton Stewart, for many years botanist of the New York Agricultural Experiment Station at Geneva, died April 24, 1946, ten years after retirement from active service. In his long and productive career he made his chief contribution in research in the field of plant pathology. His death marks the passing of another of the older generation of distinguished workers who pioneered in the study of plant diseases in America.

Professor Stewart was born, February 13, 1868, at French Creek, New York, a small village in Chautauqua County in the extreme southwestern corner of the State. He was the only child of Almeron Lyman Stewart (1842–1931) and Charlotte Eliza Hubbard (1842–1920). His father was a farmer, and his own boyhood was spent in rural surroundings. When he was still a small child his parents left the French Creek neighborhood and moved to Iowa where they settled on a small farm in Adair County near Greenfield. There he attended the public schools and, in 1888, graduated from the Adair County Normal Institute. As he held teachers' certificates from 1886 to 1890, he probably taught for a time in the local schools. He graduated from Iowa State College in 1892 with the degree of Bachelor of Science, and in 1894 received the degree of Master of Science from the same institution. He was Assistant Botanist of the Iowa Agricultural Experiment Station from 1891 to 1894. At that period he published nearly a dozen short papers, several of them in co-authorship with Professor L. H. Pammel, Botanist of the Station. They indicate that the training received under Pammel was very important in his early development, and they reveal his growing interest in plant diseases and parasitic fungi. One of his fellow students in the laboratory was George Washington Carver, the talented negro, who later was prominent for many years at Tuskegee Institute in Alabama. Stewart and he carried on some of their investigations together and, when Stewart left Iowa, Carver succeeded him as Assistant Botanist. They published a single paper jointly, reporting results obtained in inoculation experiments with a species of *Gymnosporangium*.

In December, 1894, the Board of Control of the New York Agricultural Experiment Station at Geneva called Stewart from Iowa to the position of Mycologist at the newly created Long Island Branch Station at Jamaica. From the beginning, it was understood that he was to concern himself chiefly with plant disease control. Arriving at Jamaica, he found only an improvised laboratory, located in a crowded tenement house. With meagre equipment under trying conditions, he began the career in his native State that

687

FRED CARLETON STEWART
1868–1946

was to extend over four decades. With characteristic industry and determination he soon had field experiments outlined and under way. Since in his later years he was known especially for his long-continued and diversified researches on the diseases of potatoes and their control, it is noteworthy that his first season's work on Long Island definitely established his interest in the problems of potato growing. His first bulletin on the spraying of potatoes, covering the results of his field experiments in 1896, appeared only fifteen years after the discovery of Bordeaux mixture. In those early days much remained to be determined concerning the efficacy of spray mixtures on different plants and methods of application. Stewart's other publications at that period dealt with diseases of carnations, cucumbers, and other market-garden crops. His most noteworthy accomplishment while at Jamaica was perhaps his demonstration of the bacterial nature of a newly discovered wilt disease of sweet corn, then prevalent on Long Island. His paper on the disease, including a detailed description of the cultural characters of the causal organism, appeared in 1897 when as yet only a few species of bacteria had been shown conclusively to be plant pathogens. The following year Erwin F. Smith verified his observations, called the malady "Stewart's disease of sweet corn," and named the organism *Pseudomonas stewarti*. Also, in 1899, Stewart described and figured a bacterial disease of onions without naming the organism involved. In this case many years later Burkholder, in establishing *Phytomonas alliicola*, expressed the conviction that Stewart had dealt with the same species and apparently had been the first to recognize the disease.

Stewart remained at Jamaica only three years. Then, convinced of his need for additional phytopathological training, he obtained a year's leave of absence and entered the Graduate School of Cornell University. After a very brief stay in Ithaca he decided that recent advances in techniques could best be learned in some of the European laboratories, and in the winter of 1897–1898 he spent several months in travel and study abroad. For a time he was in residence at the University of Munich in contact with Robert Hartig and Karl von Tubeuf. At the end of his first year on Long Island he had returned to Iowa on vacation, and at Bassett in Chickasaw County, November 20, 1895, had married Annie Alene Chestek, daughter of John and Amelia (Cummings) Chestek. She accompanied her husband on the trip to Europe. It was an outstandingly pleasant and profitable experience for both of them.

On returning to America, Stewart was appointed Botanist and Head of the Department of Botany of the New York Agricultural Experiment Station. He entered on his new duties at Geneva in August, 1898. In the Annual Report of the Station for 1897, Director W. H. Jordan had stated that the Botanist would "be selected with reference to his fitness to take up investigations in plant pathology." Though, in the years immediately preceding Stewart's appointment, miscellaneous matters of phytopathological nature had been handled by the Horticulturist, the large fruit and vegetable

growing interests of the State were demanding that more attention be given to plant disease control. In earlier days, soon after the Station was established, J. C. Arthur had been appointed its first Botanist, but remained at Geneva only three years, leaving in 1887 to go to Purdue University. After a lapse of eleven years the position was now revived for Stewart. It was not until 1920 that, with reorganization of the administrative work of the Station, the Department of Botany was renamed the Division of Botany, and Stewart's title became Chief in Research. Finally, in 1936, only a few weeks before his retirement from active service, the Division of Botany became the Division of Plant Pathology. In 1923, with the merging of the Station and the New York State College of Agriculture at Ithaca he had become also Professor of Botany in Cornell University, and at his retirement was made Professor Emeritus. In addition to his three years as Mycologist at the Jamaica branch station, he served the New York Agricultural Experiment Station at Geneva for thirty-eight years. At his death in 1946, at the age of seventy-eight, his years of residence in Geneva lacked only two of covering a half century.

Stewart published regularly and frequently, chiefly in the field of plant pathology. His papers totaled more than one hundred and sixty, and included more than seventy-five Station bulletins and reports. In 1902 he began an extensive series of experiments on potato spraying, and annually for ten years published a bulletin reporting his results and conclusions. In 1912, in Bulletin 349, he summarized the work for the ten-year period. This decade of experimentation on potato disease control is one of the classics of American phytopathology. Stewart became interested early in the obscure maladies of potatoes which are now known as the virus diseases. He studied their characteristic symptomatology, sought to separate them into mosaic, leaf roll, spindling sprout, and other such categories, and attempted their control by isolation of the seed plot and by roguing. He carried on many miscellaneous experiments in potato culture, and developed methods of potato disease control that became standard practices. Keeping in contact with work in progress at other stations, he shared his ideas freely with other investigators. In the early years he cooperated with Dr. L. R. Jones who at that period was also deeply interested in potato diseases in Vermont.

Though Stewart was probably best known for his long-continued and diversified studies on potatoes, he published numerous papers on diseases of many other plants. He had a predilection for assembling short notes on relatively unrelated subjects within the covers of a single bulletin. Hidden away in such bulletins will often be found valuable early records. He was an exceptionally keen observer, and was most methodical in keeping detailed notes on his field observations and activities. Each morning on reaching his office it was his routine procedure to write down carefully records of the preceding day. These notes on small uniform-sized sheets of paper were filed by him in chronological sequence for each disease. His handwriting was very attractive and wholly legible, and the entire set of notes, to which he gave meticulous care, is preserved at Geneva available for consultation.

During Stewart's long period of service at the New York Agricultural Experiment Station he made a distinguished and enviable record. He was a successful administrator, highly respected and well-liked by his associates, and his Department was outstandingly productive in research. His subordinates shared with him in its publications and at all times received full credit for their contributions. His viewpoint as a plant pathologist was definitely practical. He placed major emphasis on plant disease control, and sought earnestly to render real service to the grower. In consequence he gained popular and official support for his work, even in the early days when the economic significance of the investigation of plant diseases was not as yet generally conceded.

Professor Stewart's eminence in the field of plant pathology was given recognition by the American Phytopathological Society in its early years when it elected him its fifth President. He was a charter member of the Society and, though not especially active in its affairs, was deeply interested in all phytopathological matters. He also became a charter member of the Mycological Society of America and was elected a Fellow of the American Association for the Advancement of Science.

In 1908, when Stewart was forty, he purchased a cottage camp on Seventh Lake in the southwestern Adirondack Mountains, and throughout the following thirty-two years spent a portion of every summer there vacationing with his wife and children. The lake is circled by low mountains and closely bordered by dense forest that stretches away unbroken for miles. The camp, named by the Stewarts The Birches, stands in a secluded spot on the far shore remote from the highway, and provided a quiet retreat to which Stewart welcomed his botanical friends. In 1915, he was visited by Professor George F. Atkinson, who at that period was actively engaged in the taxonomic study of the Agaricaceae. In a ten-day period they collected and studied more than two hundred species in the surrounding woods. Atkinson returned there in the summers of 1916 and 1917, and, stimulated by his enthusiasm, Stewart's own interest in the higher Basidiomycetes was definitely aroused. He kept a list of the species identified by Atkinson, and in the following years added to it, having in mind the publication of an inclusive list of the prominent fungi of the Seventh Lake region. In 1921, Doctor Calvin H. Kauffman spent three weeks with him there busily collecting and identifying, and in 1931 the Stewarts entertained a group of well-known American mycologists who assembled to collect Agaricaceae with Doctor Jakob E. Lange of Denmark. In 1934, the second annual summer meeting of the Mycological Society of America was held at Seventh Lake with headquarters established at the Stewart camp. About forty-five mycologists and plant pathologists attended, and the Stewarts labored prodigiously to make the meeting outstandingly pleasant and successful. In other years Stewart collected alone or in the company of students of the fungi who occasionally visited him. His carefully edited list of Seventh Lake Fungi has not been published, but has been preserved in typewritten form in connection with his

herbarium. It includes about 750 species in 120 genera, most of them being Hymenomycetes. The list constitutes a noteworthy enumeration of the prominent fungi of the mixed hardwood and coniferous forest typical of northern New York. Stewart's growing interest in the mushrooms in his later years led him to publish several bulletins on a few easily recognized edible species. He also wrote a popular circular entitled "How to know the mushrooms and toadstools."

Following his retirement in 1936 Stewart spent several winters in Florida collecting leisurely and seeking rest and relaxation. The loss of his wife in the autumn of 1937 was a bitter blow and was followed by other misfortunes. The property on Seventh Lake was sold in 1941, and during his remaining years he resided at Geneva with the family of a daughter. The news of his death came as a shock to many of his old friends who had failed to keep in touch with him in his last days.

Professor Stewart was a tall slender man of erect carriage and natural dignity of manner that seemed at times to verge on austerity.[2] At heart he was a kindly person taking sincere pleasure in being helpful to others. He had a pleasant disposition, a well developed sense of humor, and thoroughly enjoyed a good joke, especially when it dealt with human interest or reaction. He was a most thoughtful host and an intensely loyal friend. The personification of trustworthiness, he was absolutely honest in thought and deed. An especially modest man, he avoided the appearance of seeking honors or distinction, and gladly made way for more aggressive contemporaries. There was never any doubt, however, as to his position on a question, and he was unwavering in the defense of his convictions. He was by temperament distinctly conservative. Premature or ill-considered actions were distasteful to him. Unsound or doubtful propaganda, designed to obtain financial backing for plant disease work lacked his support. For example, at the historic chestnut blight conference at Harrisburg, when the spread of the disease to the Appalachian forests threatened catastrophe, he opposed seeking a heavy appropriation for a spectacular program which he regarded as of doubtful expediency. He possessed too a streak of obstinacy, which found him at times supporting a lost cause long after there was any remaining hope of success. This was true of his advocacy of simplified spelling which he used in correspondence for a considerable period of years and even employed in a manuscript submitted by him as a test case to the editor of Phytopathology. He served for several years on the Advisory Council of the National Simplified Spelling Board. In all such cases, whether one agreed with him or not, there could be no question of his sincerity. One of his most noticeable characteristics was his earnestness in the detailed prosecution of any task that he undertook, even though it were relatively unimportant. His attitude toward his work was intensely serious, and steadfastness of purpose marked his days.

[2] Photograph: Mycologia 28: 99. 1936.

PUBLISHED WRITINGS

Origin of cultivated plants. Iowa Hort. Soc. Trans. 26: 443–445. 1892.

Relation of leaf structure to hardiness. Iowa Hort. Soc. Trans. 27: 466–468. 1893.

Stomata and palisade cells of leaves. Iowa Acad. Sci. Proc. 1892, 1, pt. 3: 80–84. 1893.

A key for the identification of the weed seeds found in clover seed. Iowa Acad. Sci. Proc. 1892, 1, pt. 3: 84–90. 1893.

Prevention of corn and oats smut. Iowa Agr. Exp. Sta. Bul. 20: 721–728. 1893. (With L. H. PAMMEL.)

The impurities of clover seed. Iowa Agr. Exp. Stat. Bul. 21: 805–814. 1893.

The influence of fungicides upon the germination of seeds. Agr. Sci. 8: 215–231. 1894. (With L. H. PAMMEL.)

Specific gravity and weight of wheat seeds. Iowa Agr. Exp. Sta. Bul. 25: 26–31. 1894. (With L. H. PAMMEL.)

Effects of heat on the germination of corn and smut. Iowa Acad. Sci. Proc. 1894, 2: 74–78. 1895.

Witches' brooms on cherry trees. Gard. and Forest 8: 269. 1895; same in N. Y. (Geneva) Agr. Exp. Sta. Rpt. 14: 532–533. 1896.

Inoculation experiments with *Gymnosporangium macropus* Lk. Iowa Acad. Sci. Proc. 1895, 3: 162–169. 1896; same in N. Y. (Geneva) Agr. Exp. Sta. Rpt. 14: 535–544. 1896. (With G. W. CARVER.)

Report of the Mycologist. N. Y. (Geneva) Agr. Exp. Sta. Rpt. 14: 519–546. 1896.

Combating carnation rust. N. Y. (Geneva) Agr. Exp. Sta. Bul. 100: 36–68. 1896.

Potato diseases on Long Island in the season of 1895. N. Y. (Geneva) Agr. Exp. Sta. Bul. 101: 70–86. 1896.

The cucumber flea-beetle as the cause of ''pimply'' potatoes. N. Y. (Geneva) Agr. Exp. Sta. Bul. 113: 311–317. 1896.

Report of the Mycologist. N. Y. (Geneva) Agr. Exp. Sta. Rpt. 15: 449–519. 1897.

The downy mildew of the cucumber; what it is and how to prevent it. N. Y. (Geneva) Agr. Exp. Sta. Bul. 119: 154–183. 1897.

Spraying potatoes on Long Island in the season of 1896. N. Y. (Geneva) Agr. Exp. Sta. Bul. 123: 234–259. 1897.

A bacterial disease of sweet corn. N. Y. (Geneva) Agr. Exp. Sta. Bul. 130: 423–439. 1897.

Experiments and observations on some diseases of plants. N. Y. (Geneva) Agr. Exp. Sta. Bul. 138: 627–644. 1897.

Report of the Department of Vegetable Pathology. N. Y. (Geneva) Agr. Exp. Sta. Rpt. 16: 345–433. 1897.

Plant diseases. East. N. Y. Hort. Soc. Proc. 2: 735–738. 1898.

Spraying cucumbers in the season of 1898. N. Y. (Geneva) Agr. Exp. Sta. Bul. 156: 376–396. 1898. (With F. A. SIRRINE.)

Different types of plant diseases due to a common Rhizoctonia. (Abstr.). Bot. Gaz. 27: 129. 1899; also, Amer. Naturalist 33: 216. 1899. (With B. M. DUGGAR.)

The stem-rot diseases of the carnation. (Abstr.). Bot. Gaz. 27: 129. 1899; also Amer. Naturalist 33: 216–217. 1899.

Report of committee on plant diseases. Eastern New York Horticulturist, April, 1899.

Leaf scorch of the sugar beet, cherry, cauliflower and maple. N. Y. (Geneva) Agr. Exp. Sta. Bul. 162: 165–178. 1899.

Notes on various plant diseases. N. Y. (Geneva) Agr. Exp. Sta. Bul. 164: 207–221. 1899.

A fruit disease survey of the Hudson valley in 1899. N. Y. (Geneva) Agr. Exp. Sta. Bul. 167: 275–308. 1899. (With F. H. BLODGETT.)

Common diseases and insects injurious to fruits. N. Y. (Geneva) Agr. Exp. Sta. Bul. 170: 381–445. 1899. (With S. A. BEACH and V. H. LOWE.)

Fruit diseases in the Hudson valley in 1899. East. N. Y. Hort. Soc. Proc. 4: 21–28. 1900.

An anthracnose and a stem rot of *Antirrhinum majus*. (Abstr.). Science 12: 581. 1900.

An anthracnose and a stem rot of the cultivated snapdragon. N. Y. (Geneva) Agr. Exp. Sta. Bul. 179: 105–111. 1900.

Experiments on the sulphur-lime treatment for onion smut. N. Y. (Geneva) Agr. Exp. Sta. Bul. 182: 145–172. 1900. (With F. A. SIRRINE.)

A fruit-disease survey of western New York in 1900. N. Y. (Geneva) Agr. Exp. Sta. Bul. 191: 291–331. 1900. (With F. M. ROLFS and F. H. HALL.)

Report of the committee on plant diseases. East. N. Y. Hort. Soc. Proc. 5: 27–29. 1901.

Plant diseases. West. N. Y. Hort. Soc. Proc. 46: 82–87. 1901.

The sterile fungus Rhizoctonia as a cause of plant diseases in America. N. Y. (Geneva) Agr. Exp. Sta. Bul. 186: 3–30. 1901; same in N. Y. (Cornell) Agr. Exp. Sta. Bul. 186: 51–76. 1901. (With B. M. DUGGAR.)

An epidemic of current anthracnose. N. Y. (Geneva) Agr. Exp. Sta. Bul. 199: 63–80. 1901. (With H. J. EUSTACE.)

Notes from the Botanical Department. N. Y. (Geneva) Agr. Exp. Sta. Bul. 200: 81–101. 1901. (With H. J. EUSTACE.)

The prevention of brown rot of the stone fruits. Conn. Pom. Soc. Proc. 11: 112–115. 1902.

Plant diseases. West. N. Y. Hort. Soc. Proc. 47: 52–54. 1902.

Are plant diseases hereditary? Florists' Exchange 14: 768. 1902.

Sulphur-lime treatment for carnation stem rot. Amer. Florist 19: 407. 1902.

A bacterial soft rot of certain cruciferous plants and *Amorphophallus simlense*. Science 16: 314–315. 1902. (With H. A. HARDING.)

Two unusual troubles of apple foliage. N. Y. (Geneva) Agr. Exp. Sta. Bul. 220: 217–233. 1902. (With H. J. EUSTACE.)

Potato spraying experiments in 1902. N. Y. (Geneva) Agr. Exp. Sta. Bul. 221: 235–263. 1902. (With H. J. EUSTACE and F. A. SIRRINE.)

Raspberry cane blight and raspberry yellows. N. Y. (Geneva) Agr. Exp. Sta. Bul. 226: 331–366. 1902. (With H. J. EUSTACE.)

Report of the committee on plant diseases. N. Y. State Fruit Growers' Assn. Proc. 2: 99–102. 1903. (With J. CRAIG.)

Fundamentals of plant diseases. N. Y. Dept. Agr. Report of Director of Farmers' Institutes and Normal Institutes for the Year 1903: 88–105. 1904.

Botany and plant diseases. West. N. Y. Hort. Soc. Proc. 48: 65–69. 1903.

Combating the black-rot of cabbage by the removal of affected leaves. N. Y. (Geneva) Agr. Exp. Sta. Bul. 232: 43–65. 1903. (With H. A. HARDING.)

Potato spraying experiments in 1903. N. Y. (Geneva) Agr. Exp. Sta. Bul. 241: 251–292. 1903. (With H. J. EUSTACE and F. A. SIRRINE.)

Vitality of *Pseudomonas campestris* (Pam.) Smith on cabbage seed. Science 20: 55–56. 1904. (With H. A. HARDING.)

Vitality of the cabbage black rot germ on cabbage seed. N. Y. (Geneva) Agr. Exp. Sta. Bul. 251: 177–194. 1904. (With H. A. HARDING and M. J. PRUCHA.)

Syllabus of illustrated lecture on potato diseases and their treatment. U.S.D.A. Office of Experiment Stations—Farmers' Institute Lecture No. 2: 3–30. 1904. (With H. J. EUSTACE.)

Fungi and fungous diseases. West. N. Y. Hort. Soc. Proc. 49: 52–55. 1904.

Botany and plant diseases. West. N. Y. Hort. Soc. Proc. 49: 63–65. 1904.

Recent work on plant diseases. N. Y. Dept. Agr., Bur. Farmers' Institutes and Normal Institutes, Rept. for 1904: 43–46. 1905.

Potato spraying experiments in 1904. N. Y. (Geneva) Agr. Exp. Sta. Bul. 264: 95–204. 1905. (With H. J. EUSTACE and F. A. SIRRINE.)

Potato spraying experiments in 1904. N. Y. State Dept. Agr., Bur. Farmers' Institutes, Rept. for 1904: 58–66. 1905.

Effect of certain arsenites on potato foliage. N. Y. (Geneva) Agr. Exp. Sta. Bul. 267: 263–284. 1905. (With W. H. JORDAN and H. J. EUSTACE.)

Report of the committee on plant diseases. N. Y. State Fruit Growers' Assn. Proc. 4: 30–32. 1905.

Botany and plant diseases. West. N. Y. Hort. Soc. Proc. 50: 84–87. 1905.

Recent investigations on plant diseases. N. Y. Dept. Agr., Bur. Farmers' Institutes and Normal Institutes, Rept. for 1905: 256–264. 1906.

Potato spraying experiments in 1905. N. Y. (Geneva) Agr. Exp. Sta. Bul. 279: 155–229. 1906. (With H. J. EUSTACE and F. A. SIRRINE.)

An outbreak of the European currant rust (*Cronartium ribicola* Dietr.). N. Y. (Geneva) Agr. Exp. Sta. Tech. Bul. 2: 61–74. 1906.

Report of committee on plant diseases. N. Y. State Fruit Growers' Assn. Proc. 5: 30–33. 1906.

Botany and plant diseases. West. N. Y. Hort. Soc. Proc. 51: 66–68. 1906.

Dodder in alfalfa seed. N. Y. (Geneva) Agr. Exp. Sta. Cir. 8: 1–4. 1907. (With G. T. FRENCH.)

Potato spraying experiments in 1906. N. Y. (Geneva) Agr. Exp. Sta. Bul. 290: 239–321. 1907. (With H. J. EUSTACE, G. T. FRENCH, and F. A. SIRRINE.)

Botany and plant diseases. West. N. Y. Hort. Soc. Proc. 52: 69–70. 1907.

The Sporotrichum bud-rot of carnations and the silver top of June grass. N. Y. (Geneva) Agr. Exp. Sta. Tech. Bul. 7: 83–119. 1908. (With H. E. HODGKISS.)

Troubles of alfalfa in New York. N. Y. (Geneva) Agr. Exp. Sta. Bul. 305: 331–416. 1908. (With G. T. FRENCH and J. K. WILSON.)

The control of plant diseases. N. Y. (Cornell) Agr. Exp. Sta. Bul. 252: 349–361. 1908, (With H. H. WHETZEL.)

Potato spraying experiments in 1907. N. Y. (Geneva) Agr. Exp. Sta. Bul. 307: 439–468. 1908. (With G. T. FRENCH and F. A. SIRRINE.)

Botanical investigations. A summary of botanical work at the Station from 1882 to 1907. N. Y. (Geneva) Agr. Exp. Sta. Rept. 26, pt. 3 (Twenty-fifth Anniversary Rept.): 119–162. 1908.

The principles of plant pathology. Address at Normal Institute—Dec. 1–5. 1908. N. Y. State Dept. Agr. Pamphlet (pp. 1–17), State Printer, Albany, Nov. 23, 1908.

Report of the committee on plant diseases. N. Y. State Fruit Growers' Assn. Proc. 7: 27–29. 1908.

Botany and plant diseases. West. N. Y. Hort. Soc. Proc. 53: 96–99. 1908.

Potato spraying experiments in 1908. N. Y. (Geneva) Agr. Exp. Sta. Bul. 311: 1–38. 1909. (With G. T. FRENCH and F. A. SIRRINE.)

Results of recent experiments in potato spraying. N. Y. State Dept. Agr., Bur. Farmers' Institutes, Rept. for 1908: 88–89. 1909.

The perennation of the clover dodder, *Cuscuta epithymum* Murr. Torreya 9: 28–30. 1909. (With G. T. FRENCH.)

Recent investigations on plant diseases. West. N. Y. Hort. Soc. Proc. 54: 77–81. 1909.

Potato spraying experiments in 1909. N. Y. (Geneva) Agr. Exp. Sta. Bul. 323: 17–52. 1910. (With G. T. FRENCH, S. M. McMURRAN, and F. A. SIRRINE.)

Notes on New York plant diseases, I. N. Y. (Geneva) Agr. Exp. Sta. Bul. 328: 305–404. 1910.

Plant diseases of present interest. West. N. Y. Hort. Soc. Proc. 55: 103–107. 1910.

Potato spraying experiments in 1910. N. Y. (Geneva) Agr. Exp. Sta. Bul. 338: 115–151. 1911. (With G. T. FRENCH and F. A. SIRRINE.)

A review of recent events in plant pathology. N. Y. Dept. Agr., Bur. Farmers' Institutes, Bul. 28: 76–85. 1911.

Diseases of the raspberry. N. Y. State Fruit Growers' Assn. Proc. 10: 169–171. 1911.

Some diseases of apples and pears in 1910. West. N. Y. Hort. Soc. Proc. 56: 61–64. 1911.

A comparative test of lime-sulphur, lead benzoate and bordeaux mixture for spraying potatoes. N. Y. (Geneva) Agr. Exp. Sta. Bul. 347: 77–84. 1912. (With G. T. FRENCH.)

Potato spraying experiments, 1902–1911. N. Y. (Geneva) Agr. Exp. Sta. Bul. 349: 99–139. 1912. (With G. T. FRENCH and F. A. SIRRINE.)

Can the chestnut bark disease be controlled? Pennsylvania Chestnut Blight Conference. Stenographic report, pp. 40–45. Harrisburg, 1912.

Pointed paragraphs on plant pathology. West. N. Y. Hort. Soc. Proc. 57: 107–109. 1912.

The control of certain plant diseases. N. Y. Dept. Agr. Bul. 37. Addresses at Farmers' Institutes 1911–1912, pp. 1421–1423. 1912.

An experiment on the control of currant cane necrosis by summer pruning. N. Y. (Geneva) Agr. Exp. Sta. Bul. 357: 1–10. 1913.

The persistence of the potato late-blight fungus in the soil. N. Y. (Geneva) Agr. Exp. Sta. Bul. 367: 357–361. 1913.

Cronartium ribicola and the proscription of *Ribes nigrum*. Phytopath. 3: 73. 1913. (With W. H. RANKIN.)

Pine blister rust and currant felt rust. West. N. Y. Hort. Soc. Proc. 58: 122–124. 1913.

The injurious effect of formaldehyde gas on potato tubers. N. Y. (Geneva) Agr. Exp. Sta. Bul. 369: 385–416. 1913. (With W. O. GLOYER.)

Facts relating to plant diseases. N. Y. State Dept. Agr. Circ. 91: 27–29. 1914; same in N. Y. State Dept. Agr. Bul. 62, pt. 1, Report of Farmers' Institutes, pp. 1835–1837. 1914.

Does *Cronartium ribicola* over-winter on the currant? N. Y. (Geneva) Agr. Exp. Sta. Bul. 374: 41–53. 1914. (With W. H. RANKIN).

Potato spraying experiments at Rush in 1913. N. Y. (Geneva) Agr. Exp. Sta. Bul. 379: 3–9. 1914.

The spindling-sprout disease of potatoes. (Abstr.) Phytopath. 4: 395. 1914.

Spraying. N. Y. Dept. Agr. Bul. 57: 1125–1131. 1914.

Diseases of fruit and farm crops. N. Y. Dept. Agr. Circ. 109: 58–60. 1914.

Report of the committee on plant diseases. N. Y. State Fruit Growers' Assn. Proc. 13: 31–35. 1914.

The spindling-sprout disease of potatoes. N. Y. (Geneva) Agr. Exp. Sta. Bul. 399: 133–143. 1915. (With F. A. SIRRINE.)

Potato-spraying experiments at Rush in 1914. N. Y. (Geneva) Agr. Exp. Sta. Bul. 405: 335–339. 1915.

Report of the committee on plant diseases. N. Y. State Fruit Growers' Assn. Proc. 14: 37–39. 1915.

Plant diseases. N. Y. Dept. Agr. Circ. 130: 178–183. 1916. (With M. F. BARRUS.)

The standardization of seed potatoes. N. Y. Dept. Agr. Circ. 130: 154–159. 1916.

Report of committee on plant diseases. N. Y. State Fruit Growers' Assn. Proc. 15: 33–38. 1916.

Observations on some degenerate strains of potatoes. N. Y. (Geneva) Agr. Exp. Sta. Bul. 422: 319–357. 1916.

Report of the committee on fruit diseases. N. Y. Dept. Agr. Circ. 145: 18–20. 1916.

The pine blister rust: its relation to currant rust in the Hudson valley. N. Y. State Fruit Growers' Assn. Proc. 16: 279–282. 1917.

Diseases of small fruits in the Hudson valley. N. Y. State Fruit Growers' Assn. Proc. 16: 235–240. 1917.

Report of the committee on plant diseases. N. Y. State Fruit Growers' Assn. Proc. 16: 29–34. 1917.

Apple diseases in relation to the apple grading law. West. N. Y. Hort. Soc. Proc. 62: 18–22. 1917.

Witches-brooms on hickory trees. Phytopath. 7: 185–187. 1917.

Blackheart of potatoes in storage. N. Y. State Potato Assn. Proc., 1916, 1: No. 3, 17–19. 1917.

Blackheart and the aeration of potatoes in storage. N. Y. (Geneva) Agr. Exp. Sta. Bul. 436: 321–362. 1917. (With A. J. Mix.)

The velvet-stemmed Collybia—a wild winter mushroom. N. Y. (Geneva) Agr. Exp. Sta. Bul. 448: 79–98. 1918.

The Phoma blight of red cedar. Phytopath. 8: 33–34. 1918.

Tubers within tubers of *Solanum tuberosum*. Brooklyn Bot. Gard. Mem. 1: 423–426. 1918.

Missing hills in potato fields: their effect upon the yield. N. Y. (Geneva) Agr. Exp. Sta. Bul. 459: 45–69. 1919.

Botany and plant diseases. N. Y. State Hort. Soc. Proc. 1: 19–24. 1919.

Notes on New York plant diseases, II. N. Y. (Geneva) Agr. Exp. Sta. Bul. 463: 157–188. 1919.

Report of the Committee on Botany and Plant Diseases. N. Y. State Hort. Soc. Proc. 2: 38–44. 1920.

The control of cedar rust on apples. N. Y. State Hort. Soc. Proc. 2: 205–212. 1920.

Thick vs. thin planting of potatoes. N. Y. State Potato Assn. Proc., 1919. 6: 22–23. 1920.

Experiments on the spacing of potato plants. N. Y. (Geneva) Agr. Exp. Sta. Bul. 474. 1920.

Further studies on the effect of missing hills in potato fields and on the variation in the yield of potato plants from halves of the same seed tuber. N. Y. (Geneva) Agr. Exp. Sta. Bul. 489. 1921.

Potato seed experiments: whole small tubers vs. pieces of large tubers of the same plant. N. Y. (Geneva) Agr. Exp. Sta. Bul. 491. 1922.

Spraying and dusting experiments with apples in 1922. N. Y. (Geneva) Agr. Exp. Sta. Circ. 63. 1922. (With P. J. Parrott and H. Glasgow.)

Fruit disease problems of today. N. Y. State Hort. Soc. Proc. 68: 61–67. 1923.

The relation of moisture content and certain other factors to the popping of popcorn. N. Y. (Geneva) Agr. Exp. Sta. Bul. 505. 1923.

Spraying and dusting experiments with apples in 1923. N. Y. (Geneva) Agr. Exp. Sta. Circ. 70. 1923. (With P. J. Parrott and H. Glasgow.)

Report of Committee on Crown Gall Inspection. *In* The Crown Gall Resolution, pp. 1–4. 1924. Printed and distributed by the American Assn. of Nurserymen, Charles Sizemore, Secretary, Louisiana, Mo. (With others.)

Inspection of nursery stock for crown-gall. N. Y. State Hort. Soc. Proc., 1924: 105–108. 1924.

Recommendations for the improvement of official inspection for crown-gall. Phytopath. 14: 172–173. 1924. (With others.)

Dusting vs. spraying. *In* Experiments with potatoes. N. Y. (Geneva) Agr. Exp. Sta. Bul. 518: 3–29. 1924. (With P. J. Parrott.)

Row competition and border effect. *In* Experiments with potatoes. N. Y. (Geneva) Agr. Exp. Sta. Bul. 518: 30–36. 1924.

Control of leafroll and mosaic in potatoes by isolating and roguing the seed plat. N. Y. (Geneva) Agr. Exp. Sta. Bul. 522. 1924.

Spraying and dusting experiments with apples in 1924. N. Y. (Geneva) Agr. Exp. Sta. Circ. 78. 1925. (With P. J. Parrott and H. Glasgow.)

How to know the mushrooms and toadstools. N. Y. (Geneva) Agr. Exp. Sta. Circ. 82. 1925.

Spraying and dusting experiments with apples in 1925. N. Y. (Geneva) Agr. Exp. Sta. Circ. 84. 1926. (With P. J. Parrott and H. Glasgow.)

The mica ink-cap or glistening Coprinus. N. Y. (Geneva) Agr. Exp. Sta. Bul. 535. 1926.

Oedocephalum fimetarium and *Peziza vesiculosa* var. *saccata* in mushroom beds. Mycologia 19: 184–187. 1927.

Experiments with cottonseed meal in mushroom culture. N. Y. (Geneva) Agr. Exp. Sta. Bul. 546. 1927.

Is *Psalliota brunnescens* under cultivation? Mycologia 21: 41–43. 1929.

Aphids as vectors of leafroll among sprouting potato tubers. N. Y. (Geneva) Agr. Exp. Sta. Tech. Bul. 171. 1930. (With H. GLASGOW.)

Aphids on potato sprouts. N. Y. (Geneva) Agr. Exp. Sta. Circ. 119. 1931. (With H. GLASGOW.)

The relative vigor and productivity of potato plants from basal and apical sets. N. Y. (Geneva) Agr. Exp. Sta. Bul. 633. 1933.

How to know the mushrooms and toadstools. N. Y. (Geneva) Agr. Exp. Sta. Circ. 82. (Revised). 1934.

A potato seed plat roguing experiment. N. Y. (Geneva) Agr. Exp. Sta. Bul. 655. 1935.

The relative vigor and productivity of potato plants from basal and apical sets cut from tubers in different stages of sprouting. N. Y. (Geneva) Agr. Exp. Sta. Bul. 658. 1936.

The uncertain Hypholoma. N. Y. (Geneva) Agr. Exp. Sta. Bul. 666. 1936.

The relation of age and viability to the popping of popcorn. N. Y. (Geneva) Agr. Exp. Sta. Bul. 672. 1936.

The persistence of *Hypholoma incertum* about tree stumps. Mycologia 28: 445–450. 1936.

MISCELLANEOUS GREENHOUSE TESTS WITH VARIOUS SOIL FUMIGANTS FOR THE CONTROL OF FUNGI AND NEMATODES

FRANK L. STARK, JR.,[1] AND BERT LEAR[2]

Several years of experimental work and field trials are usually required to establish the merits of a new soil fumigant. However, by means of greenhouse tests it has been possible to evaluate new soil fumigants accurately in a relatively short time. This paper deals with the results obtained with various fumigants for the control of fungi and nematodes in certain greenhouse tests.

FUNGICIDAL EFFICACIES OF VARIOUS FUMIGANTS

The fungicidal efficacies of various fumigants were compared as follows. A quantity of sandy loam soil was infested with a species of *Fusarium* capable of causing damping-off of peas. The soil was thoroughly mixed and placed in one-gallon glazed crocks (6½ inches diameter, 7 inches deep). The crocks were treated by placing the desired quantity of the fumigant in a hole 3½ to 4 inches deep in the center of the crock. The hole was then closed with soil and the surface "sealed" with 100 ml. water. In one treatment formalin was also applied as a water drench mixed throughout the soil. Each treatment was replicated 4 times. Eight to fourteen days after treatment each crock was planted with 50 pea seeds (var. Thomas Laxton) by removing a 2-inch layer of soil, distributing the seeds in the crock, and replacing the soil. Proper precautions were taken to prevent contamination of the soil during this operation. The soils were then maintained at a high moisture level to permit germination of the seed and favor the occurrence of damping-off. Preliminary counts of the stands of pea seedlings were made about ten days after planting, and final counts after about 20 days. A summary of the data on final stands of pea seedlings in three experiments is presented in table 1.

Chloropicrin was by far the most fungicidal of the fumigants tested. The 10 per cent methyl bromide solutions and the DD mixture (dichloropropylene and dichloropropane) had some fungicidal effect, but at dosages sufficient for nematode control, about 1.5 ml. and 0.5 ml., respectively (Table 2), they are not effective fungicides. When compared on this basis, DD mixture would be expected to have greater fungicidal effect than the methyl bromide solutions. It made no difference whether the methyl bromide was carried in ethylene dichloride or xylene. The formaldehyde drench gave good control of damping-off, but this chemical was not effective when injected, because its high solubility in water prevents an even distribution of

TABLE 1.—*Efficacies of various soil fumigants for controlling Fusarium in the soil*

Fumigant	Dosage[a] (ml.)	Average stand of peas		
		Exp. 1 Per cent	Exp. 2 Per cent	Exp. 3 Per cent
Check	....	2.0	8.0	17.2
Steam	...:......	96.0	91.5	98.0
Chloropicrin	2.00	94.0**	....	....
	1.00	95.0**	.....	....
	0.50	94.5**	95.0**	87.0**
	0.25	... ..	85.0**	66.0**
	0.10	.... .	94.0**	84.0**
DD Mixture (crude)	4.00	... ...	....	79.6
	3.00	... ...	....	63.6
	2.00	58.5**	....	51.6
	1.00	50.5**	....	... ..
	0.50	37.5**	... ...	....
Dowfume G[c]	10.00	....	83.5**	50.4**
	7.50	..... .	77.0**	81.6**
	5.00	32.5**	73.0**	75.2**
	2.50	45.5**	... ...	... ...
	1.00	1.5	....	....
Methyl bromide-xylene (1–9)	10.00	... ..	92.0**	62.0**
	7.50	... . .	83.3**	62.4**
	5.00	29.5**	72.5**	28.8**
	2.50	50.4**	....	.... ..
	1.00	5.0	.... .	... ...
Formalin (injection)	8.00	....	40.5**	....
(drench)	8.00	....	96.0**	....
(injection)	4.00	....	27.0	... ...
(drench)	4.00	....	89.5**	....
Carbon bisulfide	8.00	..... .	... ...	15.2
	6.00	... .	... ..	6.4
	4.00	.. ...	38.5*	....
	2.00	... . .	7.0	....
Tetrachlorethane	6.00	....	... ...	23.6[b]
	5.00	... . ..	82.6	33.6*
	4.00	....	... .	31.2
	2.50	... ..	37.5*	... ..
Ethylene dichloride	6.00	2.0	....	....
	4.00	2.0	....	... .
	2.00	3.5	....	....
Ethylene dibromide	0.50	....	4.0[b]	....
	0.25	....	13.5	....
Xylene	6.00	3.5	....	....
	4.00	2.0	....	... ...
	2.00	0.0	....	....
Toluene	8.00	....	22.0	....
	4.00	....	17.0	... . ..
Dichlorethyl ether	1.50	....	....	2.0
	2.40	....	....	0.0
Dichlorisopropyl ether	2.50	....	....	0.0
Least difference required for significance	19:1	13.96	24.04	14.76
	99:1	18.64	31.92	19.56

[a] Dosage used per 1-gal. crock of soil.
[b] Injury to seeds from residual fumigant.
[c] 10 per cent methyl bromide, 67 per cent ethylene dichloride, and 23 per cent carbon tetrachloride by volume.
* Odds of 19:1—better than check.
** Odds of 99:1—better than check.

its vapor throughout the soil. The other fumigants were not effective at the dosages tested.

Observations in these and in other experiments on the numbers of weeds appearing in crocks treated with the various fumigants indicate that there is a high correlation between the fungicidal efficacy of fumigants and their ability to kill the seeds of higher plants. There is no apparent relationship, however, between the period of time that a phytotoxic concentration of a fumigant persists in the soil and its ability to kill weed seeds.

THE NEMATOCIDAL EFFICACIES OF VARIOUS FUMIGANTS

The nematocidal efficacies of various fumigants were compared as follows. A quantity of sandy loam soil was infested with macerated root-knot nematode-infested roots. This soil was thoroughly mixed, placed in 1-gallon crocks, and treated as previously described. Each treatment was replicated four times. About ten days after treatment tomato seedlings or squash seeds were planted in each crock. After these plants had grown for about 4 weeks they were carefully removed from the soil and the number of nema-

TABLE 2.—*Efficacies of various soil fumigants for controlling the root knot nematode in the soil*

Fumigant	Dosage[a] (ml.)	Average number of nematode galls on indicator plants			
		Exp. 1	Exp. 2	Exp. 3	Exp. 4
Check	.	158.8	65.7	177.3	137
Ethylene dibromide	1.00	0	....	0.0	....
	0.75	....	....	....	0
	0.50	....	....	0.0	....
	0.25	....	....	....	0
Crude DD Mixture	1.50	0.0	4.0	....	....
	1.00	1.0	0.3	....	1
Redistilled DD Mixture	1.50	0.0	0.9	....	....
	1.00	16.8	8.3	....	....
Tetrachlorethane	4.00	....	....	0.3	....
	2.00	....	....	0.3	....
Dowfume G	3.00	1.2	5.1	0.3	....
	2.50	....	....	....	2
Dichlorisopropyl ether	2.00[b]	5.8	0.0	....	....
	1.50[b]	4.0	0.0	....	....
Dichlorethyl ether	1.00[b]	0.0	0.0	....	....
	0.50[b]	0.0	0.9	....	....
	0.27	....	....	....	34
Xylene	7.00	....	....	0.0	....
	3.50	....	....	0.0	....
Ethylene dichloride	6.00	....	....	....	2
	5.00	....	....	0.3	....
1,1 Dichloroethane	5.00	....	....	1.3	....
	3.00	....	....	88.3	....
Propylene dichloride	12.00	....	....	....	40
Chloropicrin	1.00	143.4	57.6	....	293

[a] Dosage used per 1-gal. crock of soil.
[b] Toxic to plants. Impossible to get plants to grow.

tode galls on their roots counted.   The data obtained from four such experiments are summarized in table 2.

The number of galls developing on the roots of these indicator plants is a good measure of the effectiveness of the fumigants.   It should be noted, however, that when soil is very heavily infested with nematodes, plants growing in it are usually stunted and it is probable that whenever a plant is stunted, there is a reduction in the number of galls on the roots.   This may give misleading indications of the effectiveness of some of the poorer treatments and also the amount of infestation of the soil as indicated by the numbers of galls on the check plants.

Ethylene dibromide was the most powerful nematocide tested.   Long persistence of phytotoxic concentrations of this chemical in the soil may limit its widespread use as a greenhouse fumigant, but should not be a serious factor in many field applications.   DD mixture and methyl bromide solutions also gave excellent control, although considerably larger dosages were required than with ethylene dibromide.   Other fumigants such as tetrachlorethane and dichlorisopropyl ether were only moderately nematocidal and persisted in the soil for far too long a time to have practical value. Chloropicrin gave inconsistent and disappointing results, reasons for which are discussed later.

EFFECT OF THE DISINTEGRATION OF THE NEMATODE GALLS OF THE PREVIOUS CROP ON THE EFFICACY OF VARIOUS FUMIGANTS

Inconsistent nematode control was obtained with chloropicrin in the tests just described (Table 2).   Since Godfrey and others[3] and Taylor[4] indicate that complete decay of the infested roots of the previous crop is necessary for best results with chloropicrin, several experiments were conducted to establish more clearly the extent to which galls must decay for chloropicrin treatment to be most effective.   In these experiments the ability of methyl bromide and DD mixture to penetrate intact galls was also studied.   The procedure consisted of placing large nonrotted nematode galls in gallon crocks of soil, treating different crocks at various time intervals, and then testing the efficacy of the treatment by setting indicator plants in the treated soil and counting the number of nematode galls that subsequently developed on their roots.

For the first experiment 68 one-gallon crocks were filled with moist, sandy loam soil, and ten large nematode galls obtained from recently dug tomato roots were distributed in the soil in each crock.   The crocks were stacked and covered to minimize water loss from the soil.   On the day the galls were placed in the soil four crocks were treated with chloropicrin by placing 0.7 ml. of the liquid in a hole 3½ inches deep in the center of each crock.   Four

[3] Godfrey, G. H., J. Olivera, and H. Hoskins.   Increased efficiency of chloropicrin for nematode control with better confinement of the gas. Phytopath. 24: 1382–1646. 1934.
[4] Taylor, A. L.   Soil fumigation with chloropicrin for control of the root-knot nematode, Heterodera marioni. Phytopath. 33: 1166–1175. 1943.

crocks were treated in the same way using 3 ml. of a 10 per cent methyl bromide solution per crock. Following treatment the hole was closed with soil and the surface "sealed" with 100 ml. water. At the same time four nontreated crocks of soil were set aside as checks. At four-day intervals thereafter other crocks were treated in the same manner, except that 1 ml. chloropicrin was used per crock. The soil temperatures were 60° to 70° F. when treatments were made. Four crocks were left untreated at each of the 12- and 24-day intervals. Each time a treatment was made sample galls were removed from similar crocks of soil and examined to determine the extent of disintegration. On the day the galls were placed in the soil they were very firm. After four days a few galls were soft, but most of them were still fairly solid. In 8 days all of the galls were soft or mushy, and by 12 days they were easily broken apart and were becoming dry. After 16 days the galls were practically dry, consisting only of a central core and

TABLE 3.—*Effect of degree of decay of nematode galls upon the efficacy of chloropicrin and methyl bromide soil treatment*

Rotting period, in days before treatment	Average number of nematode galls on plants from treated crocks	
	Chloropicrin	Methyl bromide
0	223.0	0.5
4	100.5	0.0
8	13.0	0.0
12	177.3	0.8
16	284.3	3.3
20	12.3	2.0
24	0.0	0.3

thin layer of epidermal tissue, the subepidermal tissue being completely disintegrated. In 20 days the galls were thoroughly disintegrated, with only the hard central core of the root intact.

About two weeks after each treatment a tomato seedling was set in each crock. Approximately 50 days later the plant was carefully removed from the soil and the number of nematode galls counted (Table 3).

Poor nematode control was obtained by chloropicrin treatments made on the day the galls were placed in the soil, and also after the galls had stood in the soil for only 4 days. Fairly good control was obtained by the treatment on the 8th day, but treatments on the 12th and 16th days were again ineffective. It is interesting that these poor results were obtained at these later intervals, because by the 12th day the galls were fairly well disintegrated. One possible explanation is that the nematode has a stage in its cycle which is resistant to chloropicrin and which was most prevalent 12 to 16 days after the galls were placed in the soil. The poor nematode control by the first two treatments could be attributed to poor penetration of the chloropicrin into the intact galls. Good nematode control was obtained by all the methyl bromide treatments, indicating the ability of this fumigant to

penetrate intact galls, and the high toxicity of this chemical to all stages of the root-knot nematode. The plants set in the nontreated soil were in each case so severely injured by nematodes that the number of galls could not be counted.

In order to determine whether the poor results with chloropicrin were due to the inability of the vapors to penetrate intact galls or to a resistant stage in the life cycle of the nematode, and to check on the data obtained with the methyl bromide solution, a second experiment was conducted. The soil in each of 72 crocks was infested with 10 large nonrotted nematode galls as in the previous trial. In addition, a second series of 40 crocks was infested, each with the macerated pieces of nematode galls obtained by grinding 10 large galls through a food chopper. Crocks containing whole galls and macerated galls were treated at 4-day intervals, using 1 ml. chloropicrin

TABLE 4.—*Effect of degree of decay of nematode galls and of macerating galls upon the efficacy of chloropicrin and methyl bromide soil treatments*

| Rotting period, in days before treatment | Average number of nematode galls on plants from treated crocks | | | | |
| | Chloropicrin | | Methyl bromide | Untreated | |
	Whole	Chopped[a]	Whole	Whole	Chopped
1	0.3	0.0	0.0	358.0	835.0
5	100.0	3.3	10.3	....	. ...
9	543.0	1.0	2.0	. ...	....
13	197.3	29.3	0.8	....	....
17	70.5	0.3	0.0	... .	....
21	9.0	0.0	0.0	....	. ...
25	0.0	0.0	2.0	. . . .	...
30	0.0	0.0	56.0	386.6	524.5

[a] Nematode galls ground with a food chopper before being placed in soil.

per crock, as previously described. Also, crocks containing whole galls were treated at 4-day intervals, using only 1.5 ml. of the 10 per cent methyl bromide solution. The soil temperature during storage period varied between 70° and 75° F. Three small tomato seedlings were set in each crock two weeks after treatments. After they had grown for about 5 weeks the number of galls on their roots was counted (Table 4).

Again, the efficacy of chloropicrin treatment cannot be entirely correlated with the disintegration of the galls, since excellent control was obtained by the first treatment made in crocks containing whole nonrotted galls. The galls at that time contained mostly young eggs, and only a few live larvae. Poor control was obtained by treatments made on the 5th, 9th, and 13th days. By the 9th day the galls had disintegrated and many young larvae and mature eggs were present. It appears that inability of the chloropicrin to penetrate galls is an important factor, because satisfactory control, except on the 13th day, was obtained in the crocks containing the ground inoculum. The good control on the first day in crocks containing solid galls can be explained by assuming that the nematodes at the time were in a very suscep-

tible stage and, consequently, a very low concentration of chloropicrin was sufficient to be lethal. Later when the nematodes developed to a more resistant stage, the vapor that penetrated was insufficient. These experiments would indicate that a resistant stage of the nematode, as well as poor penetration of roots may be responsible for poor results when chloropicrin treatment is made too soon after the previous crop has been removed.

The ability of methyl bromide to successfully penetrate nonrotted nematode galls was again demonstrated. No explanation can be made for the poor control obtained on the 30th day. The ability of methyl bromide to penetrate nonrotted galls is of considerable economic value. This fact enables treatment to be made as the previous crop is removed. Furthermore, the rapid dissipation of methyl bromide from the soil permits replanting shortly after treatment.

TABLE 5.—*Effect of degree of decay of nematode galls upon the efficacy of three soil treatments*

Rotting period, in days before treatment	Average number of nematode galls on plants in soil treated with			
	Chloropicrin	DD mixture	Dowfume G	Not treated
1	0.0	1.0	0.0	685
3	0.0			
5	0.25	0.0	0.25	
7	10.0			
9	2.5	0.0	0.0	
11	1.25			
13	3.5	0.5	0.5	
15	0.5			
17	0.25	1.5	8.75	
19	0.0			
21	0.0	1.25	0.75	
23	0.0			627

In a third similar experiment, the soil in each of 104 crocks was infested with 10 large firm nematode galls from tomato roots. At two-day intervals, four crocks were each treated with 1 ml. chloropicrin. At four-day intervals four more crocks were treated with 1 ml. of a 10 per cent methyl bromide solution, and four others with 0.5 ml. crude DD mixture. Each time a treatment was made sample galls were examined to determine which nematode stages were present. After 12 treatments had been made 3 squash seeds were planted in each crock. The squash plants were removed 15 days later and the nematode galls counted (Table 5).

Good nematode control was obtained by all treatments, probably because the temperature of the soil was higher than in previous experiments. Again, chloropicrin treatments made after the galls had rotted somewhat were less successful than those made soon after the galls were placed in the soil. All stages of the nematode were present when the galls were placed in the soil, but young eggs were most abundant. By the 5th day young larvae were plentiful. Most of the eggs had hatched by the 11th day. It appears, therefore, that the poorest control with chloropicrin was obtained at about

the time when the majority of the nematode eggs were reaching maturity and when the first instar larvae were well developed but had not yet broken out of the eggs.   Like methyl bromide, crude DD mixture appears to be effective regardless of the condition of the galls or nematodes.

A laboratory experiment was conducted to determine if the free-living larvae are more susceptible to chloropicrin than larvae still within the egg shell.   Two milliliters of a water suspension containing all stages of the root-knot nematode were placed in Petri dishes and 10 ml. of a chloropicrin solution, containing 1 gm. chloropicrin in 1000 gm. water, were added.   The larvae in suspension were then watched under the microscope.   All of the larvae ceased moving in less than ten minutes and were presumed to be dead. Young larvae and old larvae appeared to succumb equally rapidly.   Ten minutes after all movement ceased the suspension was diluted with 100 ml. of water.   All stages of the nematode were then removed from the solution by centrifuging and decanting and were transferred to Petri plates of 2 per cent water agar containing mercuric chloride (1 to 10,000) to prevent the growth of contaminants.   These plates were examined 24 hours later and many live free-living larvae were present.   They had undoubtedly developed from larvae that had been within their egg shells when exposed to chloropicrin.   It appears, consequently, that the larvae within the egg shell are resistant to chloropicrin.   No concrete data have been obtained regarding the relative susceptibility of young immature nematode eggs, but since excellent nematode control was obtained in several of the experiments when young eggs were most prevalent, it would appear that immature eggs are easily killed.   In similar experiments with methyl bromide solution and DD mixture no live larvae were found on the day following treatment, which would indicate that all stages of the root-knot nematode are readily killed by these chemicals.

It may be concluded from these experiments that in the case of methyl bromide or DD mixture treatments it is not necessary to delay soil treatment until galls from the previous crop have rotted.   Whereas, in the case of chloropicrin treatment it is desirable for consistent results to delay treatment for 10 to 20 days, depending upon soil temperature and moisture conditions, to allow for decay of the root galls and to permit the nematodes to develop to a susceptible stage.

TIME REQUIRED FOR METHYL BROMIDE TO PENETRATE NONROTTED NEMATODE GALLS IN THE SOIL

An indication of the rapidity with which methyl bromide penetrates nonrotted nematode galls in the soil was obtained by the following experiment. Twenty-eight one-gallon crocks of soil were each infested with ten large firm nematode galls from tomato roots.   Twenty-four of the crocks were treated by placing 1.5 ml. of a 10 per cent methyl bromide solution in a hole 3½ inches deep in the center of each crock.   The hole was closed with soil and the surface wet with 100 ml. water following treatment.   Two hours after

treatment the galls were removed from four of the crocks treated with methyl bromide and transferred to four crocks of steamed soil. Galls were transferred from other groups of four crocks to steamed soil after 4, 8, 12, 24, and 36 hours, respectively. In four crocks the galls were left in the methyl bromide–treated soil. One week after treatments 3 small tomato seedlings were set in all crocks containing the nematode galls. After 5 weeks these tomato plants were carefully removed from the soil and the number of nematode galls on their roots counted (Table 6).

It appears that under the conditions of this experiment lethal concentrations of methyl bromide diffused through the soil and penetrated nonrotted nematode galls in four hours, and that a concentration sufficient to kill a high percentage of the nematodes had penetrated in two hours. Data such as these indicate the rapidity with which soil fumigants diffuse in the soil

TABLE 6.—*Time required for methyl bromide to penetrate nonrotted nematode galls in the soil*

Time nematode galls were held in soil treated with methyl bromide[a] (hours)	Average height of tomato plants grown in soil infested with nematode galls from treated soil (inches)	Average number of galls per crock on tomato plants grown in soil infested with nematode galls from treated soil
2	15.1	16.5
4	14.0	2.3
8	14.4	1.8
12	13.8	3.5
24	13.3	1.5
36	12.9	3.0
Galls left in treated soil	12.7	0.3
Untreated	8.1	207.5

[a] Dowfume G (10 per cent methyl bromide) used for this experiment.

and emphasize the importance of having optimum soil conditions at the time the fumigants are added to the soil, rather than at some time following treatment.

CONTROL OF ORGANISMS AT VARIOUS LEVELS IN THE SOIL

In addition to establishing the merits and limitations of various fumigants, greenhouse tests can be of value in obtaining fundamental information regarding the edaphic factors affecting the action of fumigants, the effect of water seal or covers, the diffusion of fumigants, etc. Two experiments dealing with the latter subject have been conducted.

In one experiment 36 one-gallon crocks of soil were each infested with 25 grams of macerated roots infested with root-knot nematode. In one-third of the crocks the inoculum was placed in a layer ½ inch below the surface of the soil, in a second group, it was placed 3½ inches deep, and in the third 6½ inches deep, i.e., ½ inch from the bottom of the crock. Four of each group were treated by injecting 1 ml. chloropicrin 3½ inches deep in the center of each crock. Four of each group were treated with 1.5 ml. of a 10 per cent methyl bromide solution. The remaining crocks were left untreated. The

soil was lightly watered immediately following treatment. Ten days later 3 tomato seedlings were set in each crock. After growing for 5 weeks, they were carefully removed and the number of nematode galls on their roots counted. The data obtained (Table 7) indicate that poorest control was obtained in those crocks in which the nematodes were placed only $\frac{1}{2}$ inch below the surface of the soil. The chloropicrin treatment was relatively less effective at the surface than the methyl bromide.

The relatively large number of galls that formed on the roots of plants set in nontreated soil in which the inoculum had been placed $6\frac{1}{2}$ inches deep is of interest. The nematodes in these cases must have migrated up from the bottom of the crock, since practically all of the galls were on the older roots near the surface. This supports the belief that nematodes are attracted to plant roots, and it appears that the stimulus involved is active over a distance of at least several inches.

TABLE 7.—*Effect of depth of nematodes in the soil on the efficacy of chloropicrin and methyl bromide soil fumigation*

Treatment[a]	Depth of inoculum in soil (inches)	Average number of nematode galls per crock
None	0.5	294.8
	3.5	138.0
	6.5	115.0
Chloropicrin	0.5	37.3
	3.5	0.8
	6.5	0.0
Methyl bromide	0.5	3.3
	3.5	0.3
	6.5	0.5

[a] One ml. chloropicrin or 1.5 ml. methyl bromide solution injected 3.5 inches deep.

For another experiment a quantity of macerated nematode-infested roots were thoroughly mixed into a sandy soil. The soil was then placed in 3 cylindrical containers 17.5 inches in diameter and 28 inches deep. Also, 5 large intact nematode galls were distributed near the edge of the container at each 4-inch level in the soil. The soil in one container was treated with 5 ml. chloropicrin, another with 5 ml. crude DD mixture, and a third with 12.5 ml. of a 10 per cent methyl bromide mixture by placing the fumigants in a hole $4\frac{1}{2}$ inches deep in the center of each container. The surface was lightly watered after treatment. Twelve days later the soil was removed in 2-inch layers and placed in gallon crocks. In each 2-inch layer there was sufficient soil to fill 2 crocks. The whole galls were removed from the treated soil and each placed in a 4-inch clay pot of steamed soil. Indicator plants were then grown in all lots of soil, and the nematode galls that developed on their roots were counted.

It appears (Table 8) that the maximum concentration of the fumigant develops at about the level at which the liquids are injected. Chloropicrin was generally ineffective probably because of the low dosage employed, but

it was least effective at the surface layer. Methyl bromide, on the other hand, was about as effective at the surface layer as at the application level but was poor at the lower levels. This would indicate the advisability of applying fumigants with very high vapor pressures, as methyl bromide, deeper in the soil than chloropicrin. DD mixture was effective at all levels at the dosage employed.

PERSISTENCE OF VARIOUS FUMIGANTS IN THE SOIL

Since soil fumigants employed to date are very toxic to higher plants as well as to the undesirable organisms, it is necessary to delay planting treated soil until the concentration of the fumigant has reached a noninjurious level.

TABLE 8.—*Level in the soil of maximum concentration of fumigants as indicated by their effect on the root-knot nematode*

| Depth from which soil and galls were removed (inches) | Average number of galls on indicator plants | | | | | |
| | Chloropicrin treatment | | DD Mixture treatment | | Methyl bromide treatment | |
	Soil[a]	Galls[b]	Soil	Galls	Soil	Galls
0–2	90.5	31.1	0.5	0.4	4.5	0.4
2–4	8.0		0.0		4.0	
4–6	8.0	11.6	0.0	0.2	4.0	1.0
6–8	53.5		0.0		3.5	
8–10	15.5	34.6	0.0	0.0	4.5	1.8
10–12	17.5		0.0		4.5	
12–14	38.0	39.2	0.0	0.0	10.0	20.6
14–16	26.0		0.0		16.5	
16–18	29.5	32.8	0.5	0.0	32.0	18.0
18–20	77.0		1.0		18.0	
20–22	64.5	43.6	0.0	0.4	22.0	38.7
22–24	44.5		5.0		15.5	
24–26	36.0		8.0	.	16.5	
26–28	64.0	25.2	15.0	1.2	36.0	28.6

[a] Number of galls on plants grown in treated soil after whole galls were removed.
[b] Number of galls on plants grown in soil infested with whole galls taken from the treated soil.

The time required for this is of considerable importance in certain agricultural practices, particularly in greenhouse culture. Consequently, an experiment was conducted to determine the relative persistence of various fumigants in the soil.

A number of crocks were filled with moderately moist, sandy mineral soil or with muck soil. Three of these were treated with each of the dosages of the chemicals indicated in table 3 by placing the liquid in a hole 3½ inches deep in the center of each crock. Following treatment the hole was closed with soil and the surface wet with 100 ml. water. Three days prior to treatment a row of 5 wheat seeds was planted in each crock. Then, just prior to treatment a second row was planted, and at 2-day intervals after treatment additional rows were planted in each crock. The soil was kept moderately moist throughout the experiment. About 3 weeks after treatment observa-

tions were made to determine the planting day that permitted normal growth of the wheat seedlings (Table 9).

Three factors appear to be of primary importance in determining the relative persistence of phytotoxic concentrations of various fumigants in the soil: (1) the vapor pressure of the fumigant, (2) the amount of fumigant

TABLE 9.—*Persistence of toxic concentrations of various fumigants in muck and sandy soils*

Treatment	Dosage[a] (ml.)	Boiling point (degrees C.)	Relative persistence in soil[b]
Muck soil treatment			
Check	......	... .	− 3
Dowfume G	0.75	43–85	− 3
Xylene	1.50	138.8	0
DD Mixture	0.50	95–150	2
Chloropicrin	0.50	112.4	4
Sandy soil treatment			
Check	....	... ...	− 3
Methyl bromide—propylene dichloride (1–9)	0.75	43–97	0
	1.50		0
Methyl bromide-xylol (1–9)	0.75	43–138	0
	1.50		0
Methyl bromide-alcohol (1–9)	0.75	43–78	− 3
	1.50		2
Dowfume G	0.75	43–85	0
	1.50		2
Toluene	2.50	110.8	0
	5.00		2
Xylene	1.50	138.8	2
	3.00		2
DD Mixture (crude)	0.50	95–150	2
	1.00		2
Chloropicrin	0.50	112.4	2
	1.00		4
Tetrachlorethane	0.75	146.3	6
	1.50		8
Ethylene dibromide	0.25[c]	131.7	14 +
	0.50		14 +
Dichlorisopropyl ether	0.75	187.1	14 +
	1.50		14 +

[a] Amount of chemical injected per crock.
[b] Figure indicates number of days after soil treatment when wheat seed could be planted without injury. Negative values indicate days prior to treatment.
[c] Subsequent work has shown this to be an excessive dosage and consequently the period of persistence would be considerably shorter.

applied, and (3) the concentration that plants are able to tolerate. The vapor pressures of fumigants are, in general, negatively correlated with their boiling points, and the boiling point can, therefore, be used as a good criterion for judging volatility and the rapidity with which fumigants will escape from the soil. In general, fumigants that boil at about 100° to 120° C. will dissipate in a reasonable period of time, and those boiling at 130° to 140° C. or over will persist too long. However, the lower the total

dosage of the fumigant employed, and the smaller the amount applied per injection, the sooner a subtoxic concentration to plants is reached. With fumigants extremely toxic to undesirable organisms, as ethylene dibromide to nematodes, the feasibility of using very low dosages compensates for low vapor pressure to some extent. Weak toxicants, as xylene, while tolerated in higher concentrations by plants, must be employed in such high dosages for good nematode control that long aeration periods may be required. Fumigants with low boiling points and low plant toxicities, as methyl bromide, have a distinct advantage in many practices in permitting earlier planting. The ideal fumigant to control undesirable organisms in the presence of annual plants has yet to be found.

Soil conditions have a pronounced effect on the length of the aeration period. Aeration is slower in cold soils because of the reduced vapor pressure of the fumigant, and in soils having low free porosity, such as wet, heavy soils, because of poor diffusion of the fumigant. Some indication of the marked effect of soil conditions may be obtained by citing field experience with chloropicrin where in warm sandy soils, planting has been done 3 days after treatment without injury, whereas under adverse conditions injury has occurred when planting was delayed a month or more. The order in which a group of fumigants may be expected to dissipate from the soil will not be changed by soil conditions, but the relative rates may change, and in general the higher the boiling point of the fumigant, the greater the effect of adverse conditions.

DISCUSSION

It is recognized that there are certain limitations to the type of greenhouse tests herein described. However, it is believed that by means of such tests an accurate evaluation of the relative efficacies of fumigants against various organisms can be obtained. Field trials are desirable to determine the optimum dosage of a new fumigant under commercial application, but if the greenhouse tests indicate, for example, that 2 ml. of a new fumigant must be employed to achieve the same results obtainable with 1 cc. of chloropicrin, about the same relative dosages will be required under field conditions. Some variation in effectiveness may be expected, however, depending on such edaphic conditions as texture, moisture, and temperature, and in general the lower the vapor pressure of the fumigant the more it is affected by adverse soil conditions.

It may be argued that in small containers the ability of a fumigant to diffuse in the soil is not satisfactorily tested. However, since the dosage employed is decreased in proportion to the required diffusion distance, if a chemical still develops a lethal concentration throughout the container, it is safe to assume that it will diffuse the required distance in the field. That these tests will indicate the inability of a fumigant to diffuse is evident from the failure of formaldehyde when injected to control damping-off, which must be attributed to poor diffusion since good control was obtained when

this compound was mixed throughout the soil. Of course it is not possible to determine the optimum spacings or depths of application for fumigants without resorting to treating larger soil masses.

It is difficult to obtain a satisfactory evaluation of relatively nonvolatile solid materials by these tests because the thoroughness with which such chemicals are incorporated with the soil is an important factor in determining their effectiveness. The small greenhouse tests, where thorough mixing is possible, tend to overestimate the value of a compound, since in the field it is often impractical to obtain the required degree of mixing. Some standard mixing procedure should probably be employed to avoid incorporating the powder more thoroughly than can be accomplished in the field.

SUMMARY

A number of soil fumigants were compared in greenhouse experiments designed to test the merits and limitations of soil fumigants.

Chloropicrin was the most effective fungicide of the fumigants tested. DD mixture and methyl bromide solutions were also fungicidal, but only at considerably higher dosages than are required for nematode control.

Ethylene dibromide was the most efficacious nematocide, followed by DD mixture, methyl bromide solutions, and chloropicrin in descending order of effectiveness.

For consistent effective root-knot nematode control with chloropicrin it was necessary to delay treatment for 10 to 20 days following the removal of the previous crop to allow for decay of the root galls and to permit the nematodes to develop to a susceptible stage. Methyl bromide solutions and DD mixture were effective regardless of the condition of the galls. A lethal concentration of methyl bromide diffused through the soil and penetrated large intact nematode galls in 2 to 4 hours.

Maximum concentrations of fumigants developed in the soil at about the level to which they were injected. With chloropicrin treatment the surface of the soil was less effectively fumigated than were the lower levels. Methyl bromide, while about as effective near the surface as at the injection level, gave poorer results at the lower levels.

The persistence of phytotoxic concentrations of the fumigants in the soil was found to depend on the vapor pressure of the fumigant, the amount applied, and the concentration that plants are able to tolerate.

Department of Plant Pathology,
    Cornell University,
        Ithaca, New York.

THE CONTROL OF CABBAGE DOWNY MILDEW THROUGH
THE USE OF SPRAYS[1]

H. H. FOSTER

INTRODUCTION

Cabbage downy mildew (*Peronospora parasitica* (Fr.) Tul.), better known to Mississippi truck growers as "rust," occurs each year in the Copiah County trucking area, frequently causing growers severe loss. Truck farmers have considered downy mildew as the most serious cabbage disease in Mississippi. If mildew first appears during late December and weather conditions are unfavorable for rapid disease development, cabbage plants may not be killed since mildew develops slowly on older plants, but transplanting from coldframes to the field may be delayed.

Downy mildew has been an important factor in causing approximately one-half of the Copiah County cabbage acreage to be set with cabbage plants grown outside this area. The estimated ten-year average for cabbage in Mississippi is 6,940 acres; the greatest acreage to date, 8,300, was set in 1944. An efficient and economical control measure would insure individual growers of healthy, mildew-free cabbage plants.

LITERATURE REVIEW

Downy mildew has previously been reported (7) as severe in Mississippi. The disease, under southern conditions, has previously been described (2, 3) and environmental factors affecting downy mildew of cabbage have been determined (4). Eddins (2, 3) has shown that spraying is both successful and practical in Florida. In his earlier publication (2) he recommended Spergon (tetrachloro-parabenzoquinone) and Fermate (ferric dimethyl dithiocarbamate) applied as spray or dust but stated that "the sprays have proved more effective than the dusts." In a later paper (3) he limited his recommendation to Spergon applied either as a spray or dust.

Although Anderson (1) showed Fermate to be an effective fungicide for the control of downy mildew of tobacco, this organic fungicide has not been equally effective in the control of cabbage downy mildew. In his first recommendation Eddins (2) stated that "Spergon treated plants have grown more rapidly and have been freer of mildew than those sprayed or dusted with Fermate." Later he reported (3) Spergon as the only fungicide tested which was found to give satisfactory control.

Previously, mention has been made by Foster and Pinckard (5) of the control of cabbage mildew by means of benzene vapor and a separate report regarding the action of benzene vapor has been prepared (6). The present paper is limited to the control of cabbage mildew by means of sprays.

METHODS AND MATERIALS

During the past two years eight different fungicides (Table 1) have been tested in the greenhouse for the control of cabbage downy mildew. Spergon (wettable) and Dow Seed Protectant No. 5 contain the same active ingredient (tetrachloro-parabenzoquinone) though in different proportions and with different amounts of emulsifier. The spreaders Orvus and Vatsol O.T.C. were used at the rate of ½ pound per 100 gallons. Following repeated greenhouse tests the more effective fungicides were further tested under outside coldframe or seedbed conditions.

TABLE 1.—*The efficacy of fungicidal sprays, applied three times a week, in controlling downy mildew of cabbage in the greenhouse*

Fungicide[a]	Rate per 100 gal.	No. of trials	Spreader	Disease rating[b]
	Lb.			
Yellow Cuprocide	1	5	Vatsol	2
Dithane B-11[c]	1	5	Orvus	0–1
Spergon (wettable)[d]	4	5	Vatsol	0–1
Dow Seed Protectant No. 5[d]	2	5	Orvus	0–1
Dow Seed Protectant No. 6[e]	¼	3	Orvus	1–2
Fermate[f]	2	3	Vatsol	2–3
Phygon (wettable)[g]	¼	2	Vatsol	3
AO3 (wettable)[h]	1	2	Vatsol	2–3
Unsprayed control	......	8	....	3–4

[a] Fungicides donated without cost through the courtesy of the following: Rohm & Haas Company; United States Rubber Company, Naugatuck Chemical Division; E. I. du Pont de Nemours & Co., Grasselli Chemicals Department; and The Dow Chemical Company.
[b] Key for disease rating:
    0 = No fungus sporulation, no apparent spray injury.
    1 = Trace to slight sporulation and/or trace to slight spray injury.
    2 = Slight to moderate sporulation and/or slight to moderate spray injury.
    3 = Moderate sporulation, few plants killed, and/or moderate to severe spray injury.
    4 = Abundant and general sporulation, few to many plants killed, and/or very severe spray injury.
[c] Experimental material containing Disodium ethylene bis-dithiocarbamate, zinc sulfate monohydrate, and hydrated lime.
[d] Tetrachloro-parabenzoquinone.
[e] Trichlorophenol.
[f] Ferric dimethyldithiocarbamate.
[g] 2,3-dichlor-1,4-naphthoquinone.
[h] Phenanthraquinone.

In both greenhouse and seedbed experiments cabbage varieties grown commercially in this area were used, such as Golden Acre, Copenhagen Market, and Round Dutch. For greenhouse experiments cabbage seed were sown in 4-inch clay pots. Usually four to eight pots of cabbage seedlings (approximately 30 seedlings per pot) were used in testing each fungicide. An unsprayed control was used for a comparative check. Spray applications were begun shortly after the cotyledons appeared, and in advance of mildew sporulation. In greenhouse trials all plants were inoculated with a spore suspension following the third spray application. Plants were kept

shaded and moistened for approximately 24 hours following inoculation. In the greenhouse the fungus usually sporulated abundantly on the cotyledons of unsprayed (control) plants within five days following inoculation. Spray treatments were continued, usually 6 to 12 applications, until unsprayed control plants were killed or surviving control plants began to recover from the mildew disease. In the seedbed, sprays were applied until plants were of size for field transplanting, usually until they had four or five true leaves.

In early greenhouse experiments a weekly schedule of three spray applications was maintained. Later greenhouse experiments indicated that two spray applications a week with Dithane B-11, Spergon (wettable), and Dow Seed Protectant No. 5 would give practical mildew control.

Early in the spring of 1945 an experiment was designed to compare four different fungicides in an outside seedbed, applied at two different frequencies, each fungicide being applied in duplicate on one-square-yard units. Golden Acre variety was sown February 10, and final data were taken May 2 and 3, 1945. Mildew developed following combined artificial inoculation and natural infection. In the fall of 1945 three spray experiments were conducted on cabbage seedbeds sown at three different dates.

In seedbed experiments all comparable units were sown with a uniform weighed amount of seed. Data were obtained by recording number of plants for one square foot, weight of plants cut at soil line per one square foot, and weight of 100 large plants selected at random and cut at the soil line. A disease rating based on a scale of zero to four was used for both greenhouse and plantbed experiments.

PRESENTATION OF DATA

A. *Greenhouse Trials.* In repeated greenhouse trials three fungicides, Dithane B-11, Spergon (wettable), and Dow Seed Protectant No. 5 (tetra-chloro-parabenzoquinone), resulted in a very low disease rating, indicating a high degree of control. Based on Florida reports (2, 3) Spergon (wettable) was expected to give good control at three applications a week. Dow Seed Protectant No. 5, at the rate of 2 lb. to 100 gal., gave about equally good control as Spergon (wettable) at 4 lb. per 100 gal. Among the fungicides tested in the greenhouse, Dithane B-11 at 1 lb. to 100 gal. gave perhaps the best control. However, as shown in table 2, Dithane B-11 failed to give satisfactory control in outside seedbeds. Other fungicides tested gave more or less control as compared to unsprayed plants but were definitely inferior to Spergon (wettable) and Dow Seed Protectant No. 5. Dow Seed Protectant No. 6 failed to prevent sporulation, suggesting that under more severe conditions control would be inadequate. Fermate was definitely inferior in control. Two additional fungicides from U. S. Rubber Company, Phygon (wettable) and No. AO3 (wettable), induced some injury and failed to prevent sporulation. Plants in unsprayed control pots were 100 per cent infected, which resulted in abundant sporulation. Usually many control plants were killed. Results of greenhouse sprays, three applications each week, including explanation of disease rating, are summarized in table 1.

During the course of these investigations several emulsifiers and spreaders were used, certain of which were more satisfactory than others. With certain fungicides the emulsifier or spreader was of major importance. For example, Dow Seed Protectant No. 5 failed to make a satisfactory suspension when mixed directly with water or with water and certain emulsifiers such as B-1956. However with Orvus used as an emulsifier Dow No. 5 produced a finely divided suspension and proved to be an effective fungicide in the control of downy mildew. With several fungicides, including Spergon (wettable), Vatsol O.T.C. gave excellent results as an emulsifier and spreader. In preliminary tests self-emulsifying cotton-seed oil (one per cent solution) appeared markedly toxic to cabbage seedlings, inducing stunting and malformation of young cabbage plants.

B. *Seedbed Trials.* In the first seedbed experiment it was attempted to determine if two spray applications a week would give approximately as good control as sprays applied three times each week. A total of six and eight spray applications were applied to one-square-yard units receiving two and three spray applications a week, respectively. Four fungicides were used, the three fungicides giving the best control in previous greenhouse studies and Fermate. All treatments were in duplicate. In this experiment both Spergon (wettable) and Dow No. 5 induced satisfactory control. With Spergon (wettable) both stand and weight of plants were greatest when sprays were applied three times a week. However, the disease rating was the same for two spray applications a week, suggesting that irregular spacing of seed might, in part, explain the difference in results. Dow No. 5 plots had approximately the same average stand count whether they had two or three spray applications a week. Weight of plants per square foot and weight of 100 plants pulled at random was in favor of two spray applications each week. The average disease rating was the same in all treatments for Dow No. 5 and Spergon (wettable), giving additional confirmation to the high degree of control these compounds induced under greenhouse conditions. Fermate resulted in relatively poor control when compared with Dow No. 5 and Spergon (wettable), except for the number of plants per square foot following three spray applications a week. In that treatment the plant stand was rather high but plants were so small and stunted that plant weight was very low. The disease rating for both Fermate treatments was 3. Results from the unsprayed control plots show that within the two control units the total number of surviving plants per square foot were 6 and 34, respectively. The control units had a disease rating of 4. This is good evidence of an exceedingly severe mildew attack. Results of this experiment, including disease rating, are shown in table 2. This experiment strongly suggests that under severe conditions two spray applications each week of the more effective fungicides, when carefully applied, will satisfactorily control cabbage downy mildew.

Additional evidence relating to the effective control of cabbage downy mildew by spraying was obtained during the fall and winter season of

1945–46. In the first spray experiment a seedbed of approximately six square yards was sown to Round Dutch on October 15, and divided into three units; the two outer sections were sprayed with Spergon (wettable) and Orvus (emulsifier); the central section remained the unsprayed control. Cabbage mildew developed following natural infection. A total of six spray

TABLE 2.—*The efficacy of fungicidal sprays in controlling downy mildew of cabbage in the plant bed, in 1945*

Treatment	Av. no. of plants per 1 sq. ft.	Av. wt. of plants per 1 sq. ft. (above soil line)	Av. wt. of 100 plants pulled at random (above soil line)	Average disease rating[c]
		Grams	Grams	
Dithane B-11,[a]				
1 lb. to 100 gal., twice a week	127	34	31	3
Dithane B-11,[a]				
1 lb. to 100 gal., 3 times a week	137	47	41	3
Spergon (wettable),				
4 lb. to 100 gal., twice a week	243	115	67	0–1
Spergon (wettable),				
4 lb. to 100 gal., 3 times a week	274	152	86	0–1
Dow Seed Protectant No. 5,				
2 lb. to 100 gal., twice a week	226	139	87	0–1
Dow Seed Protectant No. 5,				
2 lb. to 100 gal., 3 times a week	227	132	78	0–1
Fermate,				
2 lb. to 100 gal., twice a week	155	55	42	3
Fermate,				
2 lb. to 100 gal., 3 times a week	216[b]	61	39	3
Unsprayed control	20	7	7	4

[a] Dithane B-11 was strictly experimental material and should not be confused with the more recent product, Dithane D-14.
[b] Very small weak plants.
[c] Key for disease rating:
  0 = Vigorous plant growth, no sign of infection.
  1 = Vigorous plant growth, 1 or 2 lower true leaves occasionally with trace or slight necrosis. All true leaves holding well, stems bright. No fresh fungus sporulation.
  2 = Rather vigorous growth, occasional lower true leaf with necrotic lesions and with some chlorosis, occasional lower leaf ready to drop or dropped. Little or no killing of plants in early seedling stage, stems bright. Usually no fresh sporulation.
  3 = Growth fair to poor, more or less stunting, usually a few plants killed during early seedling stage. Necrosis mostly general on true leaves, with one or more lower leaves usually dropped. Stem discoloration frequent. Fresh sporulation may or may not occur.
  4 = Marked stunting, usually many plants killed in early seedling stage. Most plants with abundant necrosis on true leaves, lower leaves usually dropped. Plants frequently weak and stems discolored. Secondary rots may be present on stems and roots. Fresh sporulation may or may not occur.

applications were applied twice a week; the first spray was applied November 2 and the final spray on November 19, 1945. Relatively high temperatures forced growth of cabbage plants to field transplanting size by November 20, 1945, when final data were taken.

Data based on a single sample taken from a unit sprayed with Spergon

(wettable) showed 280 plants per square foot, with plant tops weighing 209 grams, and tops from 100 large plants pulled at random weighed 112 grams. The disease rating was 1 to 1.5. In comparison, the unsprayed unit gave 199 plants per square foot, with plant tops weighing 78 grams, and 100 large plants pulled at random weighed 52 grams. The disease rating was 3. These results indicate that Spergon (wettable) is a markedly effective fungicide in the control of cabbage downy mildew.

The second spray experiment of the 1945–46 season was also with Round Dutch cabbage, sown on October 31, 1945. Treatments, including control, were in duplicate on units of one square yard. Cabbage beds were inoculated on November 11, and the first spray was applied November 12, 1945. Eleven sprays were applied; the final application was made on December 31, 1945. Sprays were applied twice a week but because of rain several applications were necessarily omitted. Final data were taken January 29, 1946. In this experiment Spergon (wettable) and Dow No. 5, with Orvus as an emulsifier, were compared with an unsprayed control. The unsprayed con-

TABLE 3.—*Comparison of Spergon (wettable), Dow No. 5, and unsprayed control. Data taken January 29, 1946*

Treatment	No. of plants per 1 sq. ft.	Wt. of plants per 1 sq. ft. above soil line	Wt. of 100 plants above soil line (large plants)	Average disease rating[a]
		Grams	*Grams*	
Spergon (wettable), 2 sprays a week	120	343	625	1.5
Dow No. 5, 2 sprays a week	99	259	586	1.5
Unsprayed control	93	160	412	3

[a] Disease rating. See table 2, footnote c.

trol produced 93 plants per square foot, weighing 160 grams, as compared with 99 plants weighing 259 grams and 120 plants weighing 343 grams for Dow No. 5 and Spergon (wettable), respectively. Weight of 100 plants pulled at random was markedly less for the untreated control. In this experiment Spergon (wettable) seemed to induce better plant growth, based on plant weight, than Dow No. 5. Data are presented in table 3.

The final and third experiment during the 1945–46 season was with the Golden Acre variety. A large farm bed of 66 square yards was sown with one pound of seed November 20, 1945. Previously the soil in one-half of the bed had been treated with chloropicrin. Four different sprays, Yellow Cuprocide, Spergon (wettable), Dow No. 5, and Fermate, were compared with unsprayed controls. All treatments were in duplicate on units of approximately five square yards and all units were inoculated with a spore suspension. The first sprays were applied on December 7; the plants were artificially inoculated on December 12, 1945. A total of 12 sprays were applied; several sprays were omitted because of rain. Final sprays were applied February 5, and final data were taken 13 to 15 days later. All treat-

ments were tested on both chloropicrin-treated soil and nontreated soil. Orvus, as an emulsifier, was used with all sprays. Results of this experiment are presented in table 4.

Data from this trial show that Spergon (wettable) gave effective control on both chloropicrin-treated and nontreated soil. The average number and weight of plants per square foot was in favor of chloropicrin-treated units, while weight of 100 plants pulled at random was in favor of units without soil treatment. Dow No. 5 also gave number and weight of plants per square foot in favor of units without chloropicrin soil treatment. The weight

TABLE 4.—*Comparison of four different fungicides applied twice a week in the control of cabbage downy mildew. Data taken February 18–20, 1946*

Treatment	Av. no. of plants per 1 sq. ft.	Av. wt. of plants per 1 sq. ft. (above soil line)	Av. wt. of 100 plants pulled at random (above soil line)	Average disease rating[c]
		Grams	Grams	
Yellow Cuprocide,[a] 1 lb. to 100 gal.	118	109	164	3
Yellow Cuprocide,[b] 1 lb. to 100 gal.	112	213	341	2
Spergon (wettable),[a] 4 lb. to 100 gal.	164	256	345	1.5
Spergon (wettable),[b] 4 lb. to 100 gal.	244	282	293	1.5
Dow Seed Protectant No. 5,[a] 2 lb. to 100 gal.	187	265	285	1.5
Dow Seed Protectant No. 5,[b] 2 lb. to 100 gal.	137	258	296	1.5
Fermate,[a] 2 lb. to 100 gal.	142	128	154	3
Fermate,[b] 2 lb. to 100 gal.	109	117	174	3
Unsprayed control[a]	148	91	121	3–3.5
Unsprayed control[b]	121	106	178	3

[a] Soil was not treated with chloropicrin.
[b] Soil was treated with chloropicrin.
[c] Disease rating. See table 2, footnote c.

of 100 plants pulled at random was greatest for units receiving chloropicrin soil treatment. Fermate and Yellow Cuprocide gave relatively poor control. Data for unsprayed control units indicated that plants were markedly small and weak. In comparing data from units receiving chloropicrin soil treatment the unsprayed units gave an average of 121 plants per square foot, weighing 106 grams, while units sprayed with Spergon (wettable) gave an average of 244 plants per square foot, weighing 282 grams.

Both Spergon (wettable) and Dow Seed Protectant No. 5 induced a similar pattern on leaves of cabbage seedlings. The pattern was characterized by rather narrow, long leaves with a pronounced vein clearing effect. It was first observed following several spray applications but did not appear to adversely affect the growth of the cabbage plants. Plant growth following field transplanting appeared normal.

EQUIPMENT AND COST

Eddins (3) stated that best results were obtained from sprayers delivering at least 200 lb. pressure. He considered poor control likely to result if small hand sprayers were used. Spray sufficient to cover 100 square yards of small cabbage seedlings was estimated at 3 to 4 gallons. Later when plants were 4 to 8 inches tall he considered that 7 to 12 gallons were required to cover 100 square yards.

A power sprayer delivering spray at 200 lb. or greater pressure would be desirable. However, such equipment is not considered essential. In these trials all experimental spraying was with hand and cart sprayers delivering an estimated maximum of 50 lb. pressure. From the results of these experiments the writer is convinced that under Mississippi conditions satisfactory control can be obtained with low pressure equipment providing spray applications are started in advance of sporulation, sprays are applied regularly, and the spraying is done carefully and thoroughly.

In Mississippi, cabbage is seeded by growers in covered beds or coldframes of 60 to 70 square yards. For 70 sq. yd. of small cabbage seedlings it is estimated that 4 to 7 gallons of spray should be used. The amount of spray should be increased for each application since complete coverage is essential. For large cabbage plants, shortly before transplanting, an estimated 12 to 15 gallons of spray should be applied to each 70 square yards.

Basing the cost on present retail prices, Spergon (wettable) required to spray 70 sq. yd. of cabbage seedlings 12 to 15 times would cost the grower approximately four to five dollars or about 17 cents per thousand plants, and require a total of 100 gallons of spray. A bed of 70 square yards should set at least three acres, 10,000 plants to the acre. In recent years cabbage plants have frequently sold at two to four dollars per thousand. Spraying is therefore a cheap control measure.

DISCUSSION

Experimental data bring out certain facts in regard to fungicidal control of cabbage downy mildew. Fungicides tested varied greatly in their ability to induce effective control; also the compatibility of fungicide and emulsifier was found to be highly important.

Under Florida conditions Eddins (2) first recommended Spergon and Fermate as fungicides for the control of cabbage downy mildew. Later he (3) limited his recommendation to Spergon, recommending Spergon spray at the rate of 2 lb. to 50 gallons of water. Under very severe conditions he found that mildew was not satisfactorily controlled unless plants were sprayed three times a week while in the plant bed. Under Florida conditions it was reported safe to suspend treatments when night temperatures dropped below 40° F. Immediately following a rain, spraying was essential in order to control the disease.

In both greenhouse and plant bed trials the writer found Spergon (wettable) and Dow Seed Protectant No. 5 to give satisfactory control when

applied twice a week, as shown in tables 1 and 2. Although spray applications were started in advance of fungus sporulation, Fermate proved to be an unsatisfactory spray for the control of downy mildew.

The difference in humidity and possibly other environmental conditions may, in part, explain why Spergon (wettable) applied twice a week in advance of sporulation has given good control in Mississippi, while in Florida three spray applications a week are usually required.

SUMMARY

1. Eight different fungicides were tested in the greenhouse for the control of cabbage downy mildew.

2. Spergon (wettable) at 4 lb. to 100 gallons and Dow Seed Protectant No. 5 at 2 lb. per 100 gallons were found, under greenhouse and plant bed conditions, to give practical control when applied twice a week.

3. Several emulsifiers and spreaders were tested. Orvus was found to be compatible with Spergon (wettable) and Dow No. 5, and it was an effective spreader.

4. Data were recorded by taking number and weight of plants per square foot, weight of 100 large plants pulled at random, and disease rating based on a scale from zero to four.

5. An estimated amount and cost of spray required for a 70-square-yard bed is given.

TRUCK CROPS BRANCH EXPERIMENT STATION,
CRYSTAL SPRINGS, MISSISSIPPI.

LITERATURE CITED

1. ANDERSON, P. J. Control of the blue mold of tobacco by a new spray. Science 96: 409. 1942.
2. EDDINS, A. H. Control downy mildew of cabbage with Spergon and Fermate. Fla. Agr. Expt. Sta. Press Bull. 589. 1943.
3. ——————. Protecting cabbage plant beds from downy mildew with Spergon. Proc. Fla. Hort. Soc. 1944.
4. FELTON, MATHIAS W., and J. C. WALKER. Environal factors affecting downy mildew of cabbage. Jour. Agr. Res. [U.S.] 72: 69–81. 1946.
5. FOSTER, H. H., and J. A. PINCKARD. Control of cabbage mildew by means of benzene vapor. (Abstr.) Phytopath. 34: 1000. 1944.
6. —————— and ——————. Control of cabbage downy mildew with benzene vapor. Phytopath. In press.
7. LEBEAU, F. J. Systemic invasion of cabbage seedlings by the downy mildew fungus. Jour. Agr. Res. [U.S.] 71: 453–463. 1945.

TOBACCO DOWNY MILDEW, ENDEMIC TO TEXAS AND MEXICO

FREDERICK A. WOLF[1]

Endemism of the pathogen causing the downy-mildew disease of culti-vated tobacco and the question as to the identity of this fungus have long been vexatious problems. In attempting to account for the outbreaks of downy mildew that occurred in the tobacco growing area of Georgia and Florida in 1921, and again in 1931, some have maintained that the causal fungus is in all probability indigenous to the United States. Other investi-gators have maintained, however, that the pathogen may have been intro-duced from Australia. It may be recalled that downy mildew disappeared after the first outbreak and did not recur until 1931. After the second out-break it not only persisted but soon thereafter became widely dispersed throughout other tobacco growing regions. When Godfrey (7) discovered a downy mildew on a native tobacco, *Nicotiana repanda* Willd., in the lower Rio Grande Valley in 1941, it was immediately conjectured that this region in Texas probably constituted the reservoir of inoculum for these original outbreaks on cultivated tobacco in the Georgia-Florida area.

Pathologists interested in tobacco diseases have for several years consid-ered the feasibility of eradicating downy mildew. The existence of a downy mildew on wild tobacco in the West is of primary significance in relation to success of efforts to eradicate the downy mildew of cultivated tobacco in the East. Unequivocal identification of the pathogen on wild tobacco therefore was necessary as a first step in formulating plans for eradication. Godfrey (7) was of the opinion that the downy mildew on *Nicotiana repanda* is *Peronospora tabacina* Adam, but positive identification was not possible since his collections consisted only of the sporangial stage. Because of these circumstances, the writer, in early March of the current year, made observa-tions and collections of downy mildew on *N. repanda* in Texas, and has embodied the results of these studies in the present report.

HISTORICAL REVIEW

A downy mildew on cultivated tobacco is known with certainty to have existed in Australia since 1890, and presumably it occurred there 30 to 40 years previously (10). It is native there on *Nicotiana suaveolens* Lehm. This pathogen was early identified as *Peronospora hyoscyami* de Bary. De Bary (4) first employed this name for an European parasite, in the spo-rangial stage, on *Hyoscyamus niger* L., a solanaceous species related to *Nico-*

tiana. Its oosporic stage was not found however, until some 50 years later when Bakhtin (3) described it from Russia.

In 1885, Farlow (6) observed on *Nicotiana glauca* Graham growing as an introduced species near San Diego, California, the sporangial stage of a downy mildew which he identified as *P. hyoscyami.* In the light of subsequent events it is now of unusual interest to recount that Farlow speculated on the possible consequences of the prevalence of this downy mildew by stating: "It is much to be feared that the disease (fungus) which attacks *Nicotiana glauca* may sooner or later extend to cultivated tobacco. If this happens the injury to tobacco will be very great. . . . If *N. glauca* and its parasite are once introduced into the Gulf States, the parasite might attack tobacco grown there and then pass on to Virginia and the other States where tobacco is the most important crop." A few years later Spegazzini (12) described, as occurring on *N. longiflora* Cav. growing near Buenos Aires, Argentina, an oosporic fungus that he named *Peronospora nicotianae.*

The outbreak, in 1921, of a downy mildew disease in tobacco seedbeds in the Georgia-Florida tobacco growing area was first reported by Smith and McKenney (11). They ascribed the disease to *Peronospora hyoscyami.* The collections of a downy mildew, identified as *P. hyoscyami,* on cultivated tobacco near Hallettsville, Texas, in 1906, and of one on *Nicotiana bigelovii* S. Watson in Nevada, in 1914 were mentioned in this report. The specimens gathered by Smith and McKenney during the 1921 outbreak lacked oospores, and failure of the fungus to produce oospores was employed by Clayton and Stevenson (5) as a likely explanation for the prompt disappearance of downy mildew from the Georgia-Florida area after this outbreak.

In Australia, Angell and Hill (2), as the result of inoculation experiments, found that more than a dozen species of *Nicotiana* are susceptible to attack by the downy mildew from cultivated tobacco, but that *Hyoscyamus niger* is immune. In their opinion these results cast grave doubt on identification of the tobacco downy mildew as *Peronospora hyoscyami.* The following year Adam (1) described the tobacco pathogen as a new species, *Peronospora tabacina,* on the basis of discovery that the organism possesses oospores of a kind that are strikingly different in size and in sculpturing of the walls from *P. nicotianae,* and of failure of the pathogen from tobacco to infect *H. niger,* as had been determined experimentally during the previous season by Angell and Hill (2).

The writer and his associates (16) in the first of their series of studies of tobacco downy mildew, also reported inability to secure infection on *Hyoscyamus niger* seedlings grown in a seedbed beside one in which tobacco seedlings became severely attacked by downy mildew. They concluded that the tobacco pathogen is not *Peronospora hyoscyami,* but instead is either *P. nicotianae* or some undescribed species. Spegazzini's type specimens of *P. nicotianae* were not available to us for comparison, and the report by Adam (1) in which he described *P. tabacina,* appeared while our report (16) was in press. The tobacco downy mildew, however, has been designated

P. tabacina in all subsequent reports of investigations in North America and in Australia. Moreover specimens of the sporangial stage of a downy mildew collected on *N. attenuata* Torr., in Nevada, in 1937, and others collected there in 1938, were identified as *P. tabacina* (13). Specimens on cultivated tobacco, containing oospores, were sent from Rio Grande do Sul, Brazil, in 1938 and were identified as *P. tabacina* by Wolf (15). The name *P. nicotianae*, however, was applied by Godoy and Coste (8) to the pathogen on cultivated tobacco grown in northern Argentina, in the State of Salta.

In 1943, Clayton and Stevenson (5) made critical comparative studies of 70 collections of tobacco downy mildew. Among 40 of these on cultivated tobacco, 9 were found to possess oospores. Among those collections from cultivated tobacco which did not possess oospores were specimens from the outbreak in Georgia-Florida in 1921, and one sent them from Rio Grande do Sul, Brazil. None of the remaining 30 collections involving 12 other species of *Nicotiana* was found to have oospores. These included specimens on *N. bigelovii* and *N. attenuata* from Nevada, as previously mentioned, and those on *N. repanda* sent from the Rio Grande Valley by Godfrey (7). Clayton and Stevenson (5) concluded (a) that neither sporangiophores, sporangia, nor oospores of *Peronospora tabacina* have distinctive morphologic features that could aid in specific determination, (b) that the evidence favors the view that this fungus is the only one on *Nicotiana* and (c) that *P. tabacina* is probably native to all temperate-zone regions having a native *Nicotiana* flora.

The observations of Clayton and Stevenson (5) on the paucity of oospores in collections of *Peronospora tabacina* accords with the findings of Angell and Hill (2) who noted only one collection with oospores among hundreds of collections of downy mildew on cultivated tobacco in Australia. The writer, however, does not regard the production of oospores as of rare occurrence, since he has never failed to find them in old decaying leaves in hundreds of specimens gathered from widely separated areas throughout the southeastern United States.

PRESENT OBSERVATIONS ON NATIVE TOBACCOS AND DOWNY MILDEW

The *Nicotiana* flora of the lower Rio Grande Valley, embracing the contiguous region in Mexico, includes five species. All of them are native or else were introduced and long ago escaped from cultivation. These species include *N. repanda* Willd., *N. glauca* Graham, *N. longiflora* Cav., *N. trigonophylla* Dunal., and *N. plumbaginifolia* Viv. Of these *N. repanda* seems to be the most abundant, has the widest range, and is represented in herbaria by the most numerous specimens, the first of which was collected nearly 100 years ago. The writer's collections of it, outside the Rio Grande Valley were made at Alice, Falfurrias, San Antonio, Austin, La Grange, and Hempstead. The range of this species in southern Texas can be closely approximated from figure 1. This map shows, by counties, the sites of all collections of *N. repanda*, represented by specimens in the herbaria of the University of Texas and the New York Botanical Garden.

Lesions of downy mildew on the leaves of *Nicotiana repanda* are scarce among the herbarium specimens examined, for the reasons that few collections were made at the appropriate season and that the rosette leaves are not commonly included on the specimens. Lesions resembling those produced by downy mildew, however, occurred on *N. longiflora*, collected on March 3, 1934, near Raymondville (Willacy Co.) and on *N. glauca*, col-
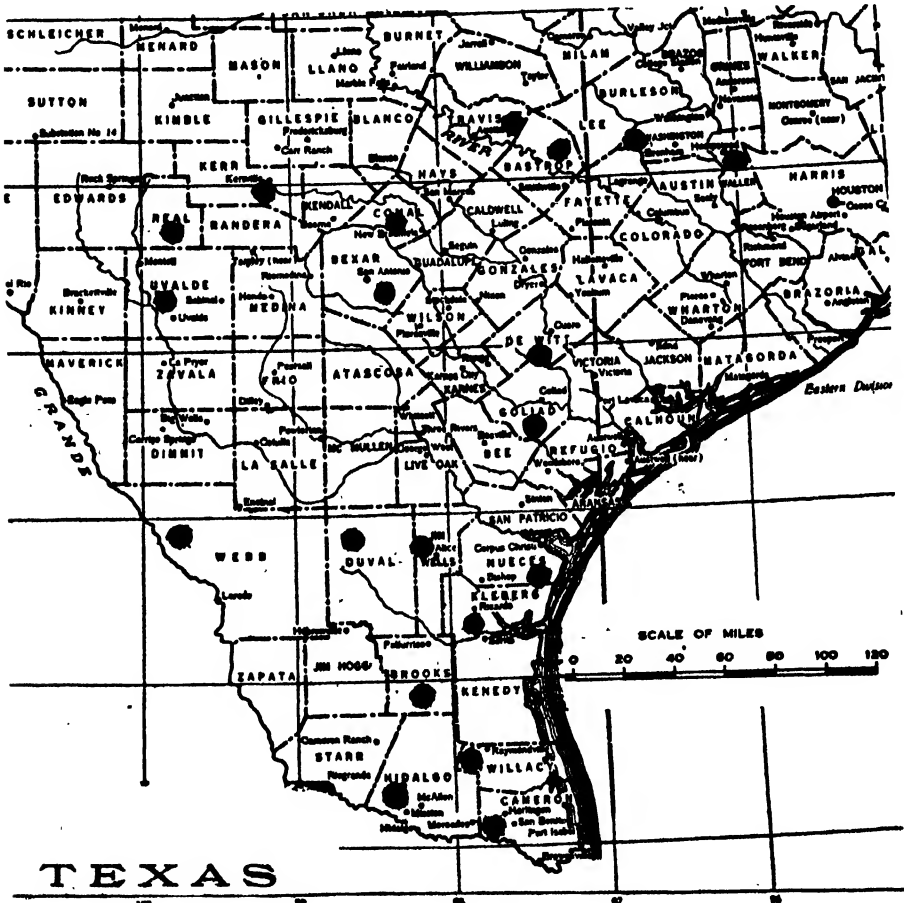
FIG. 1. Range of *Nicotiana repanda* in southern Texas. One or more specimens have been collected in each county indicated.

lected on February 9, 1942, near La Joya (Hidalgo Co.). Although these specimens were examined microscopically the cause of the lesions remains in doubt.

Present observations indicate that *Nicotiana repanda* is one of the most common and abundant weeds in citrus groves and truck gardens in the lower Rio Grande Valley, there being at times thousands of plants per acre. Plants of all ages were found, varying from young ones in the rosette stage

to those bearing flowers and mature seedpods. The flower stalks varied in height from a few inches to six or more feet, dependent upon moisture and nutrition.

Within the lower Rio Grande Valley infection by downy mildew was universal. When viewed from above, young lesions appeared as yellow, indefinite-margined areas; the lower surface of such lesions was loosely covered with a "turf" of sporangiophores. Older lesions consisted of large necrotic areas, irregularly circular, brown, and one to two centimeters in diameter. The lower surface of such lesions was usually covered with a dense grayish coating of sporangiophores. During favorable weather the

Fig. 2. Downy mildew lesions on leaves of *Nicotiana repanda*. Large necrotic lesions are produced and the leaves are deformed.

lesions enlarge by extension of the mycelium and fresh crops of sporangia were produced on this marginal zone of newly invaded tissue. Large portions of the leaf surface become necrotic and dry by confluence of lesions. As a consequence of unequal expansion of the uninvaded portions, the leaves may become curled, distorted or otherwise deformed (Fig. 2).

Lesions occurred most abundantly on the rosette leaves or other basal leaves near-by, some of which were entirely dead and decaying. When tissues from such leaves were rendered translucent by placing them in a solution of lactic acid and were examined microscopically, the old necrotic tissues were found to contain an abundance of oospores, that are structurally indistinguishable from oospores of *Personospora tabacina* occurring in decaying leaves of cultivated tobacco. Moreover their range in size accords with that of the oospores of *P. tabacina*.

Germination of sporangia was attempted since it was apparent that fresh crops of sporangia were at hand. The sporangia were suspended in tap water and kept in an icebox for approximately three hours. On being examined after this period, it was observed that germ tubes had been formed having a length of 10 to 20 times that of the sporangium.

DISCUSSION

It is now difficult or perhaps quite impossible to determine definitely the region and range within which each of the different species of *Nicotiana* is endemic. Man has unintentionally extended the range of certain of them, as indicated by Goodspeed (9), who states that the seed and capsules adhere to the coats of such animals as sheep and goats and are thus scattered locally. *N. glauca* and *N. accuminata,* for example, have become widely dispersed throughout the world. Goodspeed (9) believes the former species is native to the foothills of the Andes Mountains in northwestern Argentina, but it also has been found to occur in Australia, Egypt, the Cape Verde Islands, and has several times been introduced, with ballast, into California, Florida, and New Jersey. Moreover it has now become abundant along the lower Rio Grande.

All available evidence indicates that *Nicotiana repanda* is native to Texas and to the area in Mexico contiguous to the lower Rio Grande. It seems entirely reasonable to assume, from the observations that have been recounted, that *Peronospora tabacina* is also endemic on *N. repanda* throughout this entire region. Here as a consequence of utilization of the land for citrus and truck growing, made possible by irrigation and the use of commercial fertilizers, *N. repanda* has increased in abundance and its growth is much more luxuriant than in adjacent uncultivated areas. Under these conditions of agricultural development *P. tabacina* has coincidentally increased in amount. Moreover the severity of attack on such "cultivated" *N. repanda* has also greatly increased. An extensive reservoir of inoculum, having an area of approximately 2000 square miles, has thus been created in the lower Rio Grande Valley alone.

The survival of *Peronospora tabacina* from one season to the next, as with many other Peronosporaceae, is known to be accomplished by oospores. Apparently oospores of *P. tabacina* have not been reported previously in any wild species of tobacco, but they are produced in abundance in decaying leaves just as in cultivated tobacco. There seems no doubt that such oospores in *Nicotiana repanda* are responsible for survival of downy mildew, during summer and fall in the Rio Grande region.

It is well established that *Peronospora tabacina* is not limited to species of *Nicotiana*. The writer observed it in the Rio Grande Valley on mature plants of currant tomato, *Lycopersicon pimpinellifolium* Mill. It has been reported by various observers to occur on seedlings of tomato, *Lycopersicon esculentum* Mill.; pepper, *Capsicum annuum* L.; and eggplant, *Solanum melongena* L. Wherever *P. tabacina* occurs on these related sus-

cepts, however, the primary inoculum seems to have been derived from *Nicotiana*. The survival of this fungus for extended periods in any region is almost certainly dependent upon the existence there of species of *Nicotiana*, either native or cultivated.

Attention may be directed to the fact that nowhere in Texas is tobacco a commercial crop, although from time to time small plantings were made in the southern and eastern parts of the State. As previously mentioned, the sporangial stage of a downy mildew was collected on cultivated tobacco grown near Hallettsville, in 1906. It seems highly probable that this pathogen came from native tobacco growing near-by. Prior authentic records of endemism of *Peronospora tabacina* in southern Texas are lacking. The writer was informed, however, by Mr. H. M. Taylor, Falfurrias, Texas, that in 1892, while making a survey in the lower Rio Grande region, he noticed large brown lesions on leaves of *Nicotiana repanda*. It now seems highly probable that these lesions were induced by *P. tabacina*.

The writer (14) has previously stressed the desirability of collecting oosporic material on *Nicotiana* in the West as an aid toward solution of the problem of endemism of *Peronospora tabacina* in North America. Why this downy mildew did not earlier bridge the gap between the West and the East remains speculative, as stated by Clayton and Stevenson (5), but such speculations now have increased interest because of certain additional facts and correlated observations. A highly probable explanation of the recent introduction of downy mildew into the East is apparently provided by three interdependent circumstances. (1) Along with the agricultural development in the lower Rio Grande Valley, that first attained large proportions in the period from 1910 to 1920, there occurred coincidentally an enormous increase in abundance of *N. repanda* and its parasite, *P. tabacina*. (2) The planting of tobacco in the Georgia-Florida area was initiated in the period 1910 to 1920. As a direct consequence of the increased use of cigarettes, following World War I, and increased demand for leaf tobacco for their manufacture, there was thereafter a large increase in the acreage devoted to tobacco culture in this region. (3) Sporangia of *P. tabacina* are dispersed by air currents. Epidemiological evidence abundantly demonstrates that sporangia may be disseminated over long distances. Such evidence is supported by direct proof (17) involving the use of devices to entrap sporangia at considerable distances from their source.

With the existence of abundant inoculum in southern Texas, the prevision of tobacco seedbeds in Georgia and Florida to serve as an essential stepping-stone between Texas and the contiguous tobacco-growing areas in South Carolina, North Carolina, and Virginia, it remains only to be proved that actual transportation of sporangia over this route occurred. Such proof is not directly available. The writer believes this occurred, however, in 1921 and 1931 and can be expected to recur whenever sporangia are abundant coincidentally with protracted periods of favorable temperature, relative humidity, and cloudiness. An examination of records on file at the

U. S. Weather Bureau Station, Houston, Texas, shows that favorable periods of three to five days duration are normally frequent during January, February, and March. Prevailing surface winds are from the north and northeast although the winds aloft are prevailingly toward the northeast, a circumstance that supports the present hypothesis.

The range of *Peronospora tabacina* on native species of *Nicotiana* in Texas and Mexico is so extensive that its eradication must be regarded as impracticable. Even if it were possible to eradicate the parasite on cultivated tobacco in critical areas in the East, the likelihood of reintroduction must be considered as an ever-present threat.

A plant geographer may well raise the puzzling question as to whether *Peronospora tabacina* is to be regarded as endemic both to Australia and to North and South America, since these land masses are separated by such vast expanses of ocean. Analogous situations are not rare however among fungi. *Urnula geaster* Peck, for example, is known to occur only in Texas and in Japan; *Sarcoscypha minuscula* Boud. and Torrend, in Portugal, Bermuda, and California; *Poria cocos* Wolf, in the southeastern United States and China; and *Ophionectria cylindrothecia* Seaver, in Ohio and Bermuda. No plausible explanation of these occurrences can be offered at this time and observations such as these are difficult to harmonize with generally accepted tenets of evolution. As regards *P. tabacina* the writer is inclined to the belief that it originated in the Americas and was introduced into Australia from the Americas.

SUMMARY

This study involves the endemism and identity of a fungus causing the downy-mildew disease of cultivated tobacco.

On several native species of *Nicotiana* in Texas and Mexico, especially *N. repanda*, there occurs a downy mildew having sporangia and oospores identical with those of *Peronospora tabacina* from cultivated tobacco. All available evidence indicates that *P. tabacina* is endemic to southern Texas and the contiguous part of northern Mexico.

A reasonable explanation for the original outbreaks of downy mildew on cultivated tobacco in the East is based upon three circumstances: (1) The utilization of the lower Rio Grande region for citrus and truck crops has favored the abundance of *Nicotiana repanda* as a weed and coincidentally the growth of its parasite; (2) The planting of tobacco and the increase in acreage devoted to its cultivation in the Georgia-Florida area have provided a stepping stone in the progression of the pathogen from native tobacco in the West to cultivated tobacco in the East; (3) The organism in its sporangial stage is air-borne.

Eradication of *Peronospora tabacina* does not appear feasible or practicable and such a venture would probably result in failure.

DUKE UNIVERSITY,
DURHAM, NORTH CAROLINA.

LITERATURE CITED

1. ADAM, D. B.   Blue mould of tobacco. Jour. Dept. Agr. Victoria, **31**: 412–416. 1933.
2. ANGELL, H. R., and A. V. HILL. Downy mildew (blue mould) of tobacco in Australia. Council Sci. and Indus. Res. Bull. 65. 1932.
3. BAKHTIN, V. Note on the oospores of Peronospora hyoscyami de Bary. Mater. for Mycol. and Phytopath. **5**: 158–160. 1926 (In Russian).
4. DE BARY, A. Recherches sur le développement de quelques champignons parasites. . . . Ann. Sci. Nat. (IV) Bot. **20**: 5–148. 1863.
5. CLAYTON, E. E., and JOHN A. STEVENSON. Peronospora tabacina Adam, the organism causing blue mold (downy mildew) disease of tobacco. Phytopath. **33**: 101–113. 1943.
6. FARLOW, W. G. Notes on some injurious fungi of California. Bot. Gaz. **10**: 346–348. 1885.
7. GODFREY, G. H. Noteworthy diseases of economic crops and native plants in lower Rio Grande Valley in the spring of 1941. U. S. Dept. Agr., Pl. Dis. Reptr. **25**: 247–253. 1941.
8. GODOY, E. F., and A. D. COSTE. El "mildew" del tobaco en la region tabacalera de Salta. Revista Argentina Agron. **7**: 221–227. 1940.
9. GOODSPEED, T. H. Plant hunters in the Andes. XVI + 429 pp. Farrar and Rinehart, Inc., New York, Toronto. 1941.
10. MCALPINE, D. Report of the vegetable pathologist. Ann. Rept. Dept. Agr. Victoria (1899): 222–269. 1900.
11. SMITH, E. F., and R. E. B. MCKENNEY. A dangerous tobacco disease appears in the United States. U. S. Dept. Agr. Circ. 174. 1921.
12. SPEGAZZINI, C. Phycomycetae Argentinae. Revista Argentina Hist. Nat. **1**: 28–38. 1891.
13. STEVENSON, JOHN A., and W. A. ARCHER. A contribution to the fungus flora of Nevada. U. S. Dept. Agr., Plant Dis. Reptr. **24**: 93–103. 1940.
14. WOLF, FREDERICK A. Status of investigations of tobacco downy mildew. Phytopath. **29**: 194–200. 1939.
15. ——————. Downy mildew of tobacco in Brazil. Phytopath. **29**: 291. 1939.
16. ——————, L. F. DIXON, RUTH MCLEAN, and F. R. DARKIS. Downy mildew of tobacco. Phytopath. **24**: 337–363. 1934.
17. ——————, RUTH MCLEAN, and L. F. DIXON. Further studies on downy mildew of tobacco. Phytopath. **26**: 760–777. 1936.

CHLAMYDOSPORE GERMINATION AND ARTIFICIAL CULTURE
OF USTILAGO STRIIFORMIS FROM TIMOTHY
AND BLUEGRASS

M. J. THIRUMALACHAR AND JAMES G. DICKSON

The stripe smut caused by *Ustilago striiformis* (West.) Niessl occurs on numerous grasses and is of economic importance on bluegrass, *Poa pratensis* L. (5), timothy, *Phleum pratensis* L., and red top, *Agrostis gigantea* Roth. Failure to germinate or differences in type of germination have been reported for the chlamydospores of this fungus during the past eighty years (2). The more recent investigations (2, 3, 4, 7) have indicated considerable variation in the fungus and physiologic specialization on the grasses. Preliminary investigations by the writers have demonstrated a technique for spore germination, the wide diversity in type of development found in this species in artificial culture, and the production of chlamydospores in the several isolates from timothy comparable to those described by Leach et al (6) from bluegrass.

CHLAMYDOSPORE GERMINATION

Good germination of fresh spores was obtained with the method described by the senior author (8). Glass slides with a dried smear of spores were inverted on a rack one inch above a water surface and wet paper toweling was placed over the backside of the slides. Counts on the maximum germination were made after 2 to 10 days. In some instances individual germinating spores were transferred to potato-dextrose agar or to water agar for further study and culture; in other instances the mass of germinating spores was dispersed in melted agar and poured into Petri dishes. The spores collected in the autumn from smutted timothy and bluegrass plants in the field and placed on slides, as described, started germinating after 5 days at 65° to 72° F. and from 30 to 70 per cent had germinated after 10 days. Spores from the sori covered by the epidermis germinated 2 days earlier and with higher percentages than spores from the older sori. Fresh spores collected from smutted plants in the field in the spring or from plants in the greenhouse showed about the same high germination percentages as those collected in the autumn.

The type of spore germination was investigated in some detail, since reports (1, 3, 4, 7) have indicated a range from branching promycelial to sporidial development, with indications that the various types of germination represented variations in the biotypes of the fungus as well as in the effects of environment. Davis (1), Kreitlow (4), and Leach et al (7) described the germination of chlamydospores by the formation of branching promycelia, although Davis noticed some sporidiumlike cells. Fischer (3) reported *Ustilago striiformis* forma *hordei* as predominantly a sporidium-
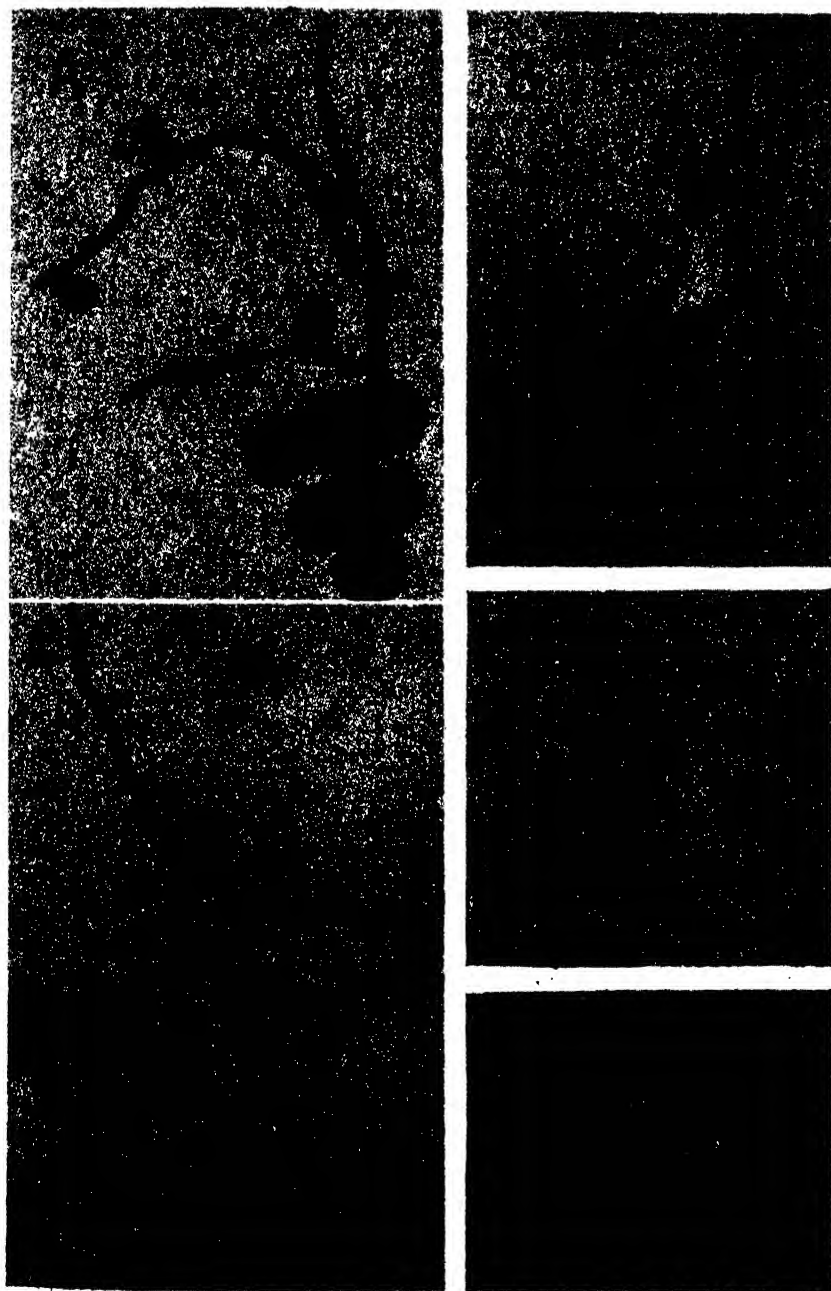
FIG. 1. Spore germination and chlamydospore formation in *Ustilago striiformis*. A. Germinating chlamydospore from timothy showing branching promycelium. × 1000. B. Same with lateral and terminal sporidia. × 1000. C. Germinating chlamydospore from bluegrass showing branched promycelium. × 1000. D. Same with sporidia. × 1000. E. Radial strands of hyphae on potato-dextrose agar, some of them rounding off into chlamydospores. × 150.

forming type. The writers have secured both sporidial and branching promycelial types of germination from the fungus on bluegrass and timothy. In the investigations in the autumn on spores from these hosts, some of the promycelia elongated either indefinitely or as much as $400\,\mu$ without branches, and others developed 2 to 3 septa and short branches (Fig. 1, A, C). The development of sporidia was not evident in these first experiments in which moisture was abundant. In germination experiments in which some of the slides were exposed to semi-dry conditions after spore germination had started, the promycelia developed septa followed by constriction of the promycelial cells into sporidiumlike structures. This was followed by lateral and terminal budding of secondary sporidia. Following this observation, individual spores were transferred to 5 per cent water agar soon after germination had started. After 4 days the promycelia fragmented and sporidia were abundant (Fig. 1, B, D). The germination of fresh spores from timothy the following spring indicated the same response to low moisture. Spores germinating in water on slides were dried after 8 days or when germination had started and were replaced in the germination chamber on 3 successive days. Later, 80 per cent of the spores so treated had produced sporidia. All isolates, however, did not show this response to drying and aeration.

CULTURE OF THE FUNGUS ON MEDIA

The fungus has been grown on culture media in either its sporidial or mycelial forms. Fischer (3) reported culturing a sporidial form of *Ustilago striiformis* forma *hordei;* Kreitlow (4) reported mycelial colonies of forma *agrostidis;* and Leach et al (6) described the complete cycle including chlamydospore formation in forma *poae-pratensis.* The latter described two distinct types of colonies: a mycelial type and a mycelial type fragmenting into sporidialike masses. Certain of the old cultures of both types produced ovate chlamydospores. The writers have obtained isolates of several types from both timothy and bluegrass. A sporidial type was obtained from spores from bluegrass that developed masses of secondary sporidia. On certain media the sporidial forms tended to produce sectors of the mycelial type (Fig. 2, B). Further investigations on the response of physiologic races are in progress. Spores from timothy produced mycelial cultures of several types as well as sporidial types (Fig. 1). The fragmenting-mycelial type produced a fine, densely interwoven mat of mycelium. Along the periphery of the colony, finely coiled hyphae abstricted small, oidiumlike [sporidiumlike according to Leach et al (6)] cells which on separation developed new colonies. Chlamydospores were formed in many of the older colonies. Thicker hyphae, easily differentiated from the fine, interwoven mycelium, developed radially with protoplasmic contents near the apices followed soon by abundant, close septations. Some hyphae produced short branches which in turn formed septa. The cells of these hyphae soon became ovate, enlarged, and thicker-walled; they finally developed into
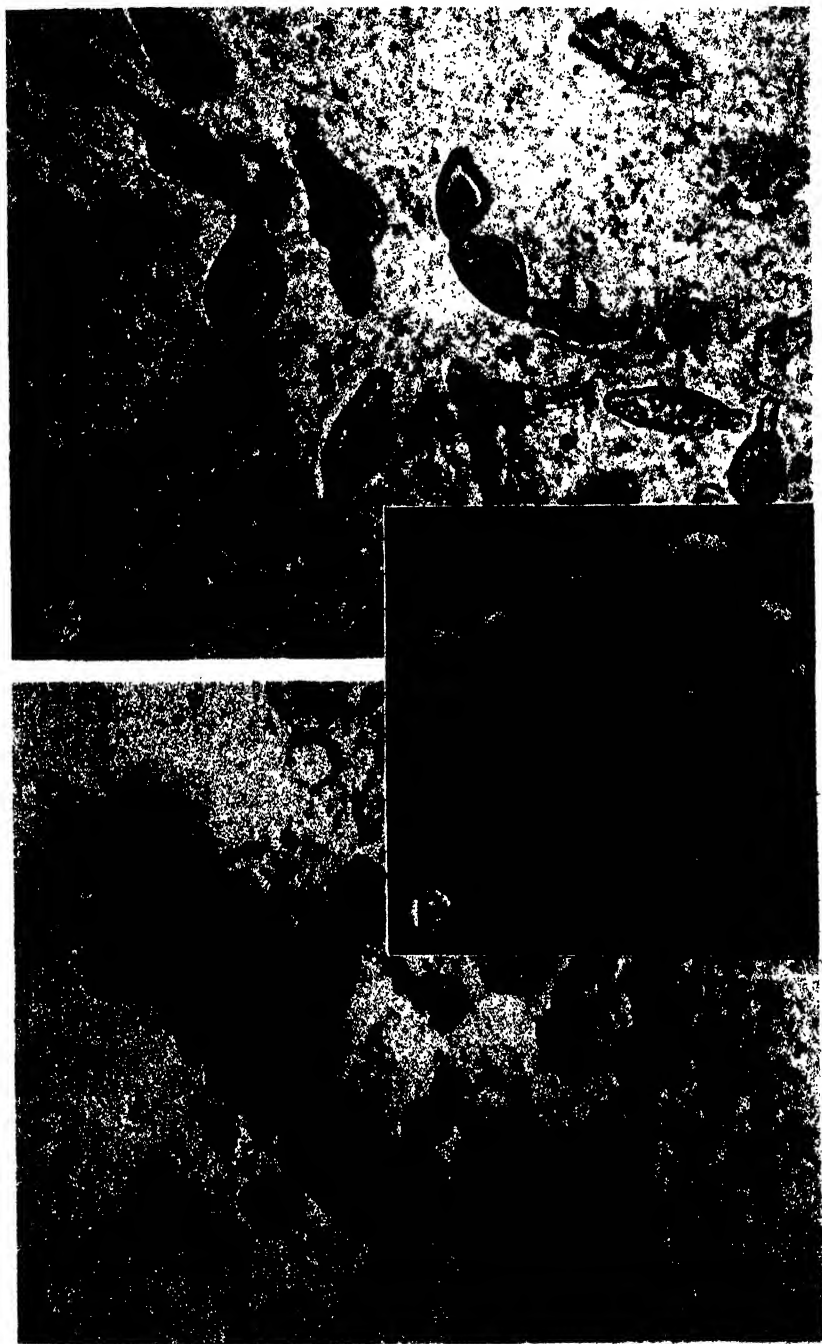
FIG. 2. Chlamydospore development and sporidial-mycelial colony of *Ustilago striiformis*. A. Ovate to lemon-shaped chlamydospores produced in artificial culture of timothy smut. ×1000. B. Sporidial colony of spores from bluegrass showing mycelial sectors along the periphery. ×1½. C. Spherical chlamydospores developed in artificial

mature spores (Fig. 1, E). The chlamydospores were usually ovate or lemon-shaped (Fig. 2, A), although one isolate produced spherical spores (Fig. 2, C). All had less marked echinulations and slightly greater size than the spores produced in host tissue. The mycelial type, described by Leach et al (7), and several other types have been isolated from the spores from timothy. Some of these remained vegetative; others produced chlamydospores in abundance. In these, small, yellowish-brown, short hyphae developed close septations; the cells became ovate; the walls thickened. The lemon-shaped spores remained attached or separated.

Good spore germination was obtained in fresh spores of *Ustilago striiformis* from bluegrass and timothy. Sporidial and mycelial types were obtained in artificial culture by single spore, sporidial, or mycelial isolations. Chlamydospore development in culture was observed in the several cultural types.

UNIVERSITY OF WISCONSIN,

MADISON, WISCONSIN.

LITERATURE CITED

1. DAVIS, W. H. Spore germination of *Ustilago striaeformis*. Phytopath. **14**: 251–267. 1924.
2. ————. Two physiologic forms of *Ustilago striaeformis*. Phytopath. **20**: 65–74. 1930.
3. FISCHER, G. W. Fundamental studies of the stripe smut of grasses (*Ustilago striaeformis*) in the Pacific Northwest. Phytopath. **30**: 93–118. 1940.
4. KREITLOW, K. W. *Ustilago striaeformis* I. Germination of chlamydospores and culture of forma *agrostidis* on artificial media. Phytopath. **33**: 707–712. 1943.
5. ————, and W. M. MYERS. Prevalence and distribution of stripe smut of *Poa pratensis* in some pastures of Pennsylvania. Phytopath. **34**: 411–415. 1944.
6. LEACH, J. G., C. V. LOWTHER, and M. A. RYAN. Stripe smut (*Ustilago striaeformis*) in relation to blue grass improvement. Phytopath. **36**: 57–72. 1946.
7. ————, and M. A. RYAN. The cytology of *Ustilago striiformis* forma *poae-pratensis* in artificial culture. Phytopath. **36**: 876–886. 1946.
8. THIRUMALACHAR, M. J. A method for germinating and staining teleutospores. Jour. Indian Bot. Soc. **19**: 71–75. 1940.

WOUND INFECTION OF OAK TREES WITH CHALARA QUERCINA AND ITS DISTRIBUTION WITHIN THE HOST[1]

B. W. HENRY[2] AND A. J. RIKER[3]

INTRODUCTION

Oak wilt has continued to encroach upon the oak population of the Upper Mississippi Valley (1, 2, 3, 6) since this disease and its causal agent, *Chalara quercina* Henry, were described (4, 5).

The introduction of the fungus into a host stem resulted in the disease, and the same organism was recovered from roots, stems, branches, twigs, and leaves of infected trees (5). These and other host-parasite relations have needed clarification as a basis for efforts to combat the disease.

This paper describes studies on different parts of the tree as infection courts and on the distribution of the pathogen within the host during disease development.

MATERIALS AND METHODS

Studies on different parts of the host as infection courts for the pathogen were made on black oak (*Quercus velutina* Lam.) woodland trees at 3 locations in Wisconsin and on red oak (*Q. borealis* Michx. f.) seedlings in the greenhouse at Madison, Wisconsin. Some bur oak (*Q. macrocarpa* Michx.) and white oak (*Q. alba* L.) woodland trees were stem-inoculated. There was no oak wilt within ¼ mile of the field plots. The trees ranged in size from greenhouse seedlings and woodland seedlings and sprouts 18-inches high to 6-inch (d.b.h.) trees 30 feet tall in their natural habitat.

The woodland trees were inoculated in the roots, stems, branches, twigs, or leaves during the period of June 16 to July 13, 1943, and results were obtained by early September, 1943. The greenhouse seedlings were inoculated in roots, stems, or leaves during April, 1942 or 1943, and the data were recorded later each summer. Root inoculations were made after scraping the soil from around a desired lateral root (tap root in the case of seedlings), and after washing the root surface with distilled water followed by 95 per cent alcohol. A culture of the pathogen on cornmeal was placed in a diagonal knife cut in the vascular tissue. The wound was covered with moist absorbent cotton and the soil was replaced. In some cases the pathogen was mixed with the soil around a tree and the roots were wounded or left unwounded. Stem and branch inoculations were

[2] Formerly Instructor in Plant Pathology, now employed at Camp Detrick, Frederick, Maryland.

[3] Professor of Plant Pathology.

made in a cut made with a chisel through at least two annual rings (5). One root, stem, or branch inoculation was made per tree. Twig, petiole, and leaf-blade inoculations were made by holding the selected part for one minute in a spore suspension and stabbing it in two or three places with a sharp, sterilized scalpel (dissecting needle in the case of leaf blades). The leaf midribs of some seedlings were injected in two places with a spore suspension. One or two twigs, petioles, or leaf blades, respectively, were inoculated per tree.

Four isolates of *Chalara quercina* were used to inoculate the woodland trees. Two of the four (C and E) were the same isolates used previously (5), except for an additional passage through the host. Isolate C, a single-spore culture originally from black oak, was used for all the leaf-blade, petiole and twig inoculations and for some root and stem inoculations on black oak. Isolate E, a single-spore culture originally from bur oak, was used for stem inoculations on black, bur, and white oaks and for some root inoculations on black oak. Isolate I, a mass culture from bur oak, and isolate J, a mass culture from red oak, were used for stem inoculations on black and bur oaks. Isolates A, C, D, E, F, G, and H (5), plus isolate I, two isolates from black oak, and one from red oak were used to inoculate seedlings in the greenhouse.

An inoculated tree was considered to be infected when foliage symptoms developed, and *Chalara quercina* was reisolated from a branch.

The distribution of the pathogen was studied within naturally diseased and stem-wound-inoculated woodland trees that showed symptoms in 1941, 1942, or 1943. The 38 naturally diseased trees, from various locations in Wisconsin, were all black and red oaks except 1 tree and 1 sprout clump of white oak. They were from 1 to 22 inches d.b.h. and 10 to 70 feet high. Twenty-six of them were used to study the vertical distribution of *Chalara quercina*, 1 of the 26 plus 9 others to study the distribution in the leaves, and 3 additional trees, all red oak, to study radial distribution in the stems. The 27 stem-wound-inoculated trees were all black oaks of sprout origin in a plot near Madison and ranged from 1 to $3\frac{1}{2}$ inches d.b.h. and 11 to 25 feet high. Six were used to study the vertical distribution of *C. quercina*, 1 of the 6 plus 2 others to study radial distribution in the stems, 1 of the 6 plus 4 others to study distribution in the leaves, and 1 of the 6 plus 15 others to study distribution in relation to foliage symptoms. The presence of the pathogen in a particular part of an infected tree was determined by isolation according to described methods (5).

WOUNDS AS INFECTION COURTS FOR CHALARA QUERCINA

Inoculation of Woodland Trees. Different parts of 173 black oak woodland trees were wound-inoculated with *Chalara quercina* to determine some of the possible portals of entry for the pathogen. Some stem-inoculated bur and white oaks were included in the plots. The 3 geographical locations, the size of the trees, and the 4 isolates of *C. quercina* had no apparent effects on the results. A summary is given in table 1.

The time between inoculation and the first-noticed symptoms varied extensively between individual trees. It was influenced perhaps by the place of inoculation. The incubation periods according to the place of inoculation were: root, 21 to 48 days; ground level, 55 to 82 days; breast height, 19 to 50 days (for bur oak, 49 to 51 days); branch, 26 to 55 days; twig, 17 to 48 days; petiole, 48 to 70 days; and for the one positive case of leaf blade inoculation, 55 days. There was some error in recording the longer incubation periods; it was impractical daily to observe widely scattered trees; thus symptoms were well advanced in some cases when first noted. The shorter periods (17 to 26 days) were fairly accurate; symptoms were noted when only a few leaves had wilted.

Characteristic symptoms developed regardless of where the trees were inoculated. The first symptoms on those inoculated in the roots or stems

TABLE 1.—*Results from wound-inoculating different parts of woodland oak trees with Chalara quercina*

Host species	Part of tree inoculated						
	Root	Stem		Branch	Twig	Leaf	
		Ground level	Breast height			Petiole	Blade
Black	8/3[a]	7/4	123/46	8/3	8/8	6/4	13/1
Bur	... ...	....	30/5	....	... ..	... .	... .
White	.... ..	.. ....	5/0	.. .. .	. .. ..	... .	..:. ....
Controls (black)							
Wounded, uninoculated	2/0	....	4/0	1/0	....	3/0	..._....
Unwounded, inoculated	3/0	....	. ..	....	6/0	6/0	6/0

[a] Numerator is the number of trees inoculated in the respective part. Denominator is the number of trees which developed symptoms and from which *C. quercina* was isolated.

appeared on scattered branches in the upper half of the crown. On the branch-, twig-, and leaf-inoculated trees, the first symptoms appeared, respectively, on the inoculated part. The symptoms soon spread throughout the crown in all cases. Branches were collected during this "intermediate" symptom stage for reisolation of the causal fungus. The collections usually were made as far away from the inoculation point as possible, particularly of branch, twig, and leaf inoculations. The terminal leader was taken in several cases. *Chalara quercina* was isolated from all inoculated trees which showed symptoms, except one stem-inoculated black oak.

The extent of symptom development by September 2 to 14, 1943, when the last notes were taken, varied between individual trees; there was little, if any, relation to the point of inoculation. At least 70 days had elapsed since all the root-, branch-, twig-, and leaf-inoculated trees and many of the stem-inoculated ones had been treated. With the exception of 2 ground-level-inoculated, 2 petiole-inoculated, and some of those stem-inoculated in July, all positive trees had shown symptoms at least 30 days previously. From

50 to 100 per cent of the primary leaves on trees of this group were dead, and from 0 to 98 per cent had fallen. Secondary leaves on suckers along the stem and main branches appeared on only 1 (root-inoculated) tree. Those trees on which symptoms had not been noted prior to the last observation date were, of course, less severely affected.

Wounds into the wood or vascular tissues of roots, stems, branches, twigs, or leaves of black oak evidently served as infection courts for *Chalara quercina.* The number of trees used in all except the breast-height studies was relatively small; thus, the relative effectiveness of the different infection courts must be considered accordingly. However, the twig inoculations appeared the most effective since 100 per cent of such treated trees became diseased. Leaf petiole was the next most effective infection court with 66 per cent positive cases. Ground-level inoculations were slightly less effective with 57 per cent; root, breast-height, and branch inoculations were equally good with 37 per cent, and leaf-blade inoculations were the least effective with 8 per cent positive results. Perhaps the penetration of the vascular elements by the causal fungus was more nearly assured by the method used for twig and petiole inoculations than by that for root, stem, and branch inoculations. Why the stem inoculations in this series of trials were only about one-half as successful as those previously reported (5) remains unexplained.

Comparatively few positive cases of disease resulted from inoculations on bur oak. The results were completely negative on white oak; this indicates further their relative tolerance (5).

None of the unwounded-inoculated or wounded-uninoculated controls became diseased.

Inoculation in the Greenhouse. In conjunction with the field studies on infection courts for *Chalara quercina,* 123 red oak seedlings growing in the greenhouse were inoculated into the roots, stems, or leaves. The results were as follows: of 11 unwounded seedlings in infested soil, 0 was infected; of 9 with wounded roots in infested soil, 0 was infected; of 15 wound-inoculated in the roots, 4 were infected; of 70 wound-inoculated in the stem, 16 were infected; of 9 injected in the midrib of the leaf blade, 1 was infected; of 9 stabbed in the leaf blade, 0 was infected. None of the 17 appropriately wounded and uninoculated controls became infected. *C. quercina* isolates A, C, D, E, F, and G gave the positive results.

The symptoms developed like those on woodland-inoculated trees. The incubation period was 14 days (2 root-inoculated seedlings) to 48 days for all except the one positive leaf inoculation; it required 103 days.

The results indicated (1) that wounds in the roots, stems, or leaves of red oak seedlings could serve as infection courts for *Chalara quercina,* (2) that infection from contaminated soil was not promising, (3) that infection from root and stem inoculations was less than that obtained with woodland trees, and (4) that infection resulting from leaf inoculations was about equally poor in both instances (Table 1).

Cross Inoculation. The presence or absence of host specificity in isolates of a pathogen bears a vital relation to the epidemiology and control of a disease. Isolates of *Chalara quercina* from black, red, bur, and scarlet oaks were pathogenic to black oaks, and one isolate from black oak was pathogenic to white oak (5). Current data confirm some of these results and demonstrate that isolates from bur and red oak may infect bur oak and that isolates from bur, black, red, and scarlet oak may infect red oak seedlings. Though some isolates failed to cause infection on red oak seedlings, there was no indication of host specificity. Isolate E, from bur oak, was pathogenic to black, red, and bur oaks but did not cause infection in any of the five white oaks inoculated (Table 1). This could be a case of host specificity, but such a conclusion would not be justifiable since it is the only instance and is based on only five individuals. Though cross inoculation studies should be carried further, the preponderance of evidence to date indicates the absence of host specificity in isolates of *C. quercina*.

DISTRIBUTION OF CHALARA QUERCINA WITHIN THE HOST[4]

Vertical Distribution. The occurrence of *Chalara quercina* in the roots, stems, and branches of infected trees has been reported (5). Information was sought on the relative frequency of the pathogen's occurrence in the different parts of the host. Isolations were made from the branches and roots of 26 naturally infected red, black, and white oak and 6 inoculated black oak trees, and from the twigs and stems of some of them. All the twigs and branches used for isolations had typical foliage symptoms.

The results were as follows: For the naturally infected trees, of the 26 root isolations, 15 were positive; of the 8 stem isolations, 7 were positive; of the 26 branch isolations, 26 were positive; of the 10 twig isolations, 8 were positive; and for the inoculated trees, of the 6 root isolations, 3 were positive; of the 6 stem isolations, 6 were positive; of the 6 branch isolations, 6 were positive; and the one twig isolation was positive. Thus, the pathogen was present in the branches of all the trees tested, in the twigs and stems of nearly all, and in the roots of over one-half of them. The host species did not seem to influence the vertical distribution of the pathogen.

Root isolations from the 6 inoculated trees and from 10 (7 red and 3 black oaks) of the 26 naturally infected trees were made in late winter or early the next summer following the appearance of foliage symptoms.

Chalara quercina was obtained from the roots of 3 of the inoculated trees and 7 (5 red and 2 black oaks) of the naturally infected trees. Thus, it may overwinter in the roots of red and black oaks.

Radial Distribution in the Stems. The radial distribution of *Chalara quercina* in the stems of infected oak trees was studied by dissecting out and culturing the desired portions of wood from three naturally infected red oaks and three stem-inoculated (August, 1941) black oaks. Symptoms were

first noted on the naturally infected trees in August and September, 1942. Isolations were made from trees Nos. 1, 2, and 3 (Table 2) on October 21, October 27, and September 3, 1942, respectively. The black oaks had partially leafed out and then completely wilted during the summer of 1942, forming no summer wood. Isolations were made about 18 inches below the inoculation point in July and August, 1942. The results of isolations from the spring and summer wood of the 1942, 1941, and 1940 annual rings of the 6 trees are given in table 2.

In naturally infected trees, *Chalara quercina* extended toward the center of the stem as far as the 2-year-old summer wood. The fungus was found to extend slightly farther, *i.e.*, to the 2-year-old spring wood, in inoculated trees. With the exception of tree No. 5, the pathogen was present in a relatively solid band of wood.

TABLE 2.—*Radial distribution of Chalara quercina in the stems of 6 oak trees that wilted in 1942*[a]

Tree no.	Type infection	Species	D.B.H. (inches)	Age and type of wood tested					
				Current year		1-year-old		2-year-old	
				Summer	Spring	Summer	Spring	Summer	Spring
1	natural	red	10[b]	0	+	+	0	0	
2	do	do	10[b]	+	+	+	0	0	0
3	do	do	20	...	+	+	+	+	0
4	inoculated	black	5	...	0	+	+	+	...
5	do	do	3½	...	+	0	+	0	0
6	do	do	2½	...	+	+	+	+	+

[a] Positive isolation of *C. quercina* is indicated by a plus sign (+) and failure to isolate it is indicated by a zero (0).
[b] Estimated.

Distribution within the Leaves. Since *Chalara quercina* sometimes inhabited oak leaves (5), further studies were made on the occurrence of the pathogen in different parts of leaves both with and without symptoms.

Isolations were made from parts of 34 leaves from 10 naturally diseased red and black oaks and 5 inoculated black oaks. All leaves were picked from wilting trees except 4 symptom-leaves that were picked from the ground soon after falling—2 beneath a naturally infected tree and 2 beneath an inoculated tree. Leaves with and without symptoms and sections of bronzed and green blade tissue were used. The petioles from all leaves and portions of the midribs, of lateral veins, and of blade tissues from some of the leaves were cultured. Results were similar with leaves from red and black oaks, so the data in table 3 are not separated according to hosts.

Chalara quercina was present most often in the petioles of diseased leaves, about half as frequently in the midribs and lateral veins, and seldom in the green portions of the blades. It was not found in the bronzed portions of blade tissues. The distribution of the pathogen in a representative

TABLE 3.—*Distribution of Chalara quercina in leaves from infected red and black oak trees*

Type infection	Symptoms	Part of leaf cultured				
		Petiole	Midrib	Lateral vein	Blade	
					Green portion	Bronzed portion
Natural	progressive bronzing	20/14[a]	15/5	9/5	15/2	12/0
Do	none	5/1	4/0	2/0	4/0	.. ..
Inoculated	progressive bronzing	8/5	5/1	6/0	5/0	4/0
Do	none	1/1	1/0	... ..	1/0	... .

[a] Numerator is the number of leaves cultured in the respective part. Denominator is the number of leaves from which *C. quercina* was isolated from the respective part.

leaf is illustrated in figure 1, B. It was isolated from the petioles but..not from other parts of 2 of the 6 leaves that showed no symptoms. The petioles and no other parts of the 4 symptom-leaves taken from the ground beneath wilting trees also yielded *C. quercina*. It may be concluded that the pathogen is likely to be present in partially bronzed leaves and may be present in leaves that show no symptoms.

Distribution in Relation to Symptoms. In relation to the possibility of control of oak wilt by pruning or sanitation measures, it is pertinent
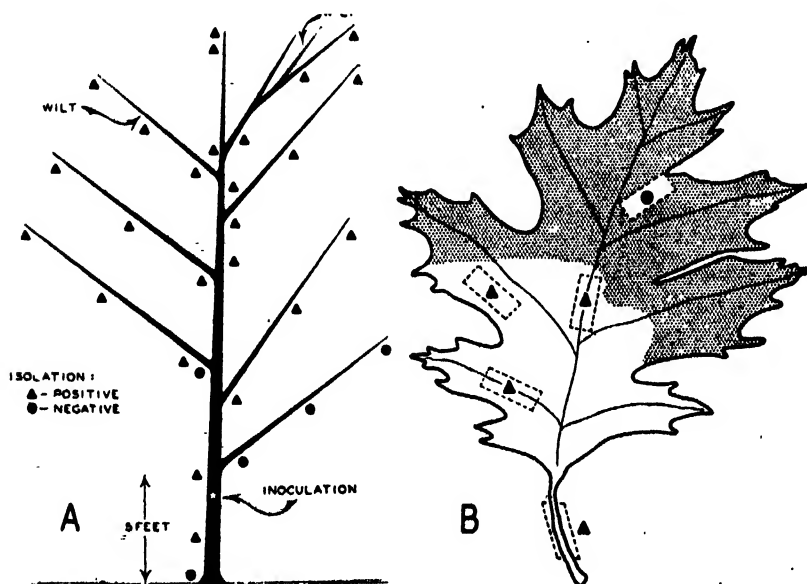
FIG. 1. Distribution of *C. quercina;* the results of isolations are indicated. A. Stem-inoculated black oak tree felled the first day symptoms were noted (24 days after inoculation). Two branches had wilted foliage; others appeared normal. B. Leaf picked from a naturally infected red oak tree; stippled, apical portion was bronzed; basal portion was green.

to know the distribution of the causal fungus in relation to symptoms. Such a study was carried out with 16 stem-inoculated black oaks. Soon after the appearance of symptoms, samples were taken from branches with wilting leaves and from other branches that appeared healthy. *Chalara quercina* was isolated from symptom and non-symptom branches of all the 16 trees.

Three of the trees were felled as soon as symptoms were noted, *i.e.*, 24, 36, and 38 days after inoculation, respectively. From 36 to 56 separate isolations were made per tree from 2 inches above the ground line to and including the current year's terminal in the apex of the crown. The distribution of *Chalara quercina* in a representative tree is shown in figure 1, A. The pathogen was isolated from the terminal shoots, most of the main branches, and the stems of each of the 3 trees. It was present in the stems to within 2 inches of the ground line in 2 of the trees and to within 39 inches in the other. The latter was felled 24 days after inoculation, or 12 days sooner than the others. Of the total 132 isolations from the 3 trees, 107 yielded *C. quercina* and 25 were sterile. The negative isolations were mostly from the lower parts of the stems. Thus, by the time symptoms were evident, *C. quercina* was well distributed throughout symptom, as well as non-symptom, branches.

DISCUSSION

These results help to clarify some of the host-parasite relations and are applicable to finding the means of disease spread and control.

Since isolates of *Chalara quercina* from one oak species can infect other species, any diseased tree is apparently a source of inoculum for neighboring oaks. White and bur oaks may be particularly important in this respect as they survive longer in a diseased condition than the red and black oaks.

The presence of the fungus in the 2-year-old wood of red and black oaks does not necessarily mean that such trees were infected 2 years before, as no infected tree in this group has been known to survive for 2 years. However, the roots of red and black oaks may be possible sources of inoculum for some time, as they are the last parts to die and have been shown to harbor the pathogen overwinter.

Wounds apparently are important for infection. Since wounds in roots, stems, branches, twigs, and leaves of black oak trees may serve as infection courts for *Chalara quercina,* one suspects, for example, insects, birds, rodents, root grafts, and tools as disseminating agents. Detached leaves may be a contributing factor since some of them harbor the pathogen. However, rain and surface water seem unpromising until evidence appears that the pathogen sporulates on the surface of infected parts. The means of dissemination is obscure but must be relatively ineffective; otherwise, such a virulent pathogen would diminish the oak population much more rapidly.

Certain common measures seem unlikely to lead to successful control.

Since rapidly growing and slowly growing large and small trees on various sites may succumb to wilt (5), control through fertilization and irrigation carries little promise. Because the fungus was found distributed throughout the crowns of infected trees, even in branches and leaves not yet showing symptoms, pruning is eliminated as a counter measure.

Sanitation measures, including the killing or removal of diseased roots, might prove valuable for control in localized areas. Dietz and Barrett (3) reported limited control over a 2-year period by sanitation methods.

SUMMARY

Typical oak wilt developed and *Chalara quercina* was reisolated from black oak woodland seedlings and sprouts and from red oak greenhouse seedlings all wound-inoculated in vascular tissues of the roots, stems, branches, twigs, and leaves.

Various isolates of the pathogen did not appear host-specific among bur, red, and black oaks.

Chalara quercina was distributed throughout the crowns of wilting trees including non-symptom branches and leaves. Roots in over one-half the trees tested were diseased.

The fungus was isolated from the current-year, 1-year-old, and 2-year-old wood of the stems of wilting trees.

DEPARTMENT OF PLANT PATHOLOGY,

   UNIVERSITY OF WISCONSIN,

     MADISON, WISCONSIN.

LITERATURE CITED

1. BRETZ, T. W. Finding of oak wilt in Missouri. U. S Dept. Agr., Plant Dis. Rptr. **28**: 951. 1944.
2. DENNING, J. A. New locations and new host records for oak wilt in Missouri. U. S. Dept. Agr., Plant Dis. Rptr. **30**: 32. 1946.
3. DIETZ, S. M., and J. W. BARRETT. Spread and control of oak wilt. (Abstr.) Phytopath. **36**: 397. 1946.
4. HENRY, B. W. *Chalara quercina* n. sp., the cause of oak wilt. Phytopath. **34**: 631–635. 1944.
5. ————————, C. S. MOSES, C. AUDREY RICHARDS, and A. J. RIKER. Oak wilt: its significance, symptoms, and cause. Phytopath. **34**: 636–647. 1944.
6. HONEY, E. E. Oak wilt and other diseases in Wisconsin. U. S. Dept. Agr., Plant Dis. Rptr. **28**: 951. 1944.

EVIDENCE OF FUSION BODIES FROM UREDIOSPORE GERM TUBES OF CEREAL RUSTS ON NUTRIENT-SOLUTION AGAR

H. A. RODENHISER AND ANNIE M. HURD-KARRER

INTRODUCTION

When urediospores of cereal rusts were germinated on nutrient-solution agar round bodies formed on the ends of some of the germ tubes. Many of these bodies fused with neighboring hyphae and the process of fusion sometimes resulted in what appeared to be extensive anastomosing unlike anything heretofore described, so far as the writers are aware. The present paper describes the "fusion bodies," the anastomosing, and some of the factors affecting their development.

MATERIALS AND METHODS

Several different kinds of media and various concentrations of mineral salts and glucose were tested in the course of the work. A satisfactory medium contained mineral salts and glucose in the following concentrations:

M/5 Magnesium sulphate	2.5	cc.
M/2 Ammonium nitrate	0.5	cc.
M/2 Calcium nitrate	1.5	cc.
M/5 Potassium acid phosphate	1.5	cc.
M/5 Dipotassium phosphate	2.5	cc.
0.5 per cent Ferric tartrate	0.5	cc.
Glucose	50	gm.
Distilled water to make	1000	cc.
Agar	20	gm.

This medium was autoclaved for 10 minutes at 12 lb. pressure. Its reaction after autoclaving was near pH 6.0.

Urediospores of the rusts listed below were dusted by means of a camelshair brush on the surface of freshly poured nutrient-solution agar about 2 mm. thick in small Petri dishes. They were incubated overnight in darkness at approximately 13° C.

Detailed studies were made with *Puccinia graminis tritici* Eriks. and E. Henn. race 56 and *P. triticina* Eriks. (*P. rubigo-vera tritici* (Eriks. and E. Henn.) Carleton) race 105; corroborative observations were made on *P. coronata avenae* Fraser and Led., *P. hordei* Otth (*P. simplex* (Koern.) Eriks. and E. Henn.), *P. dispersa* Eriks. and E. Henn., and *P. sorghi* Schw.

DEVELOPMENT OF FUSION BODIES AND NETWORKS

Urediospores incubated overnight in darkness on nutrient-solution agar containing 5 per cent glucose produced a profuse growth of mycelium, often
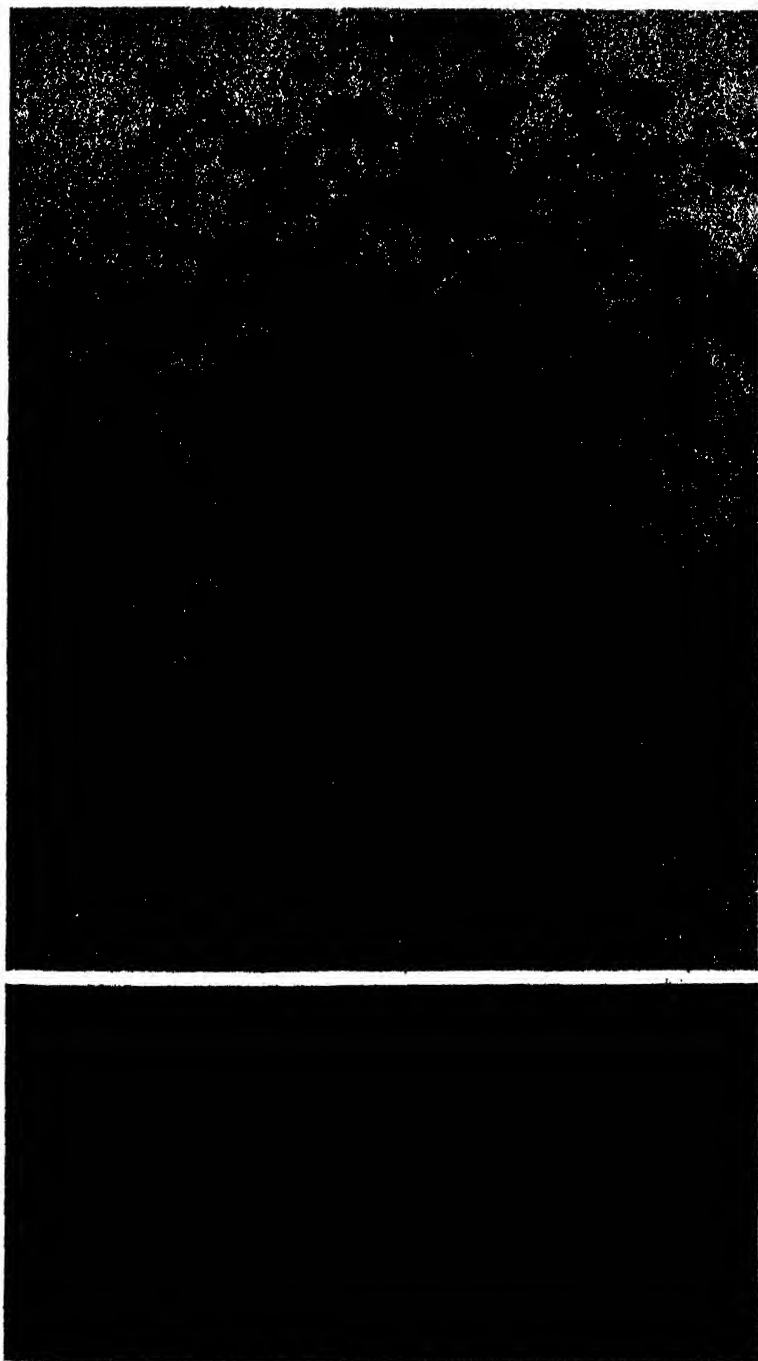
FIG. 1. Networks of hyphae of *Puccinia tritioina*, with fusion bodies: A, On nutrient-solution agar; B. On surface of inoculated leaf of Little Club wheat. × 300.
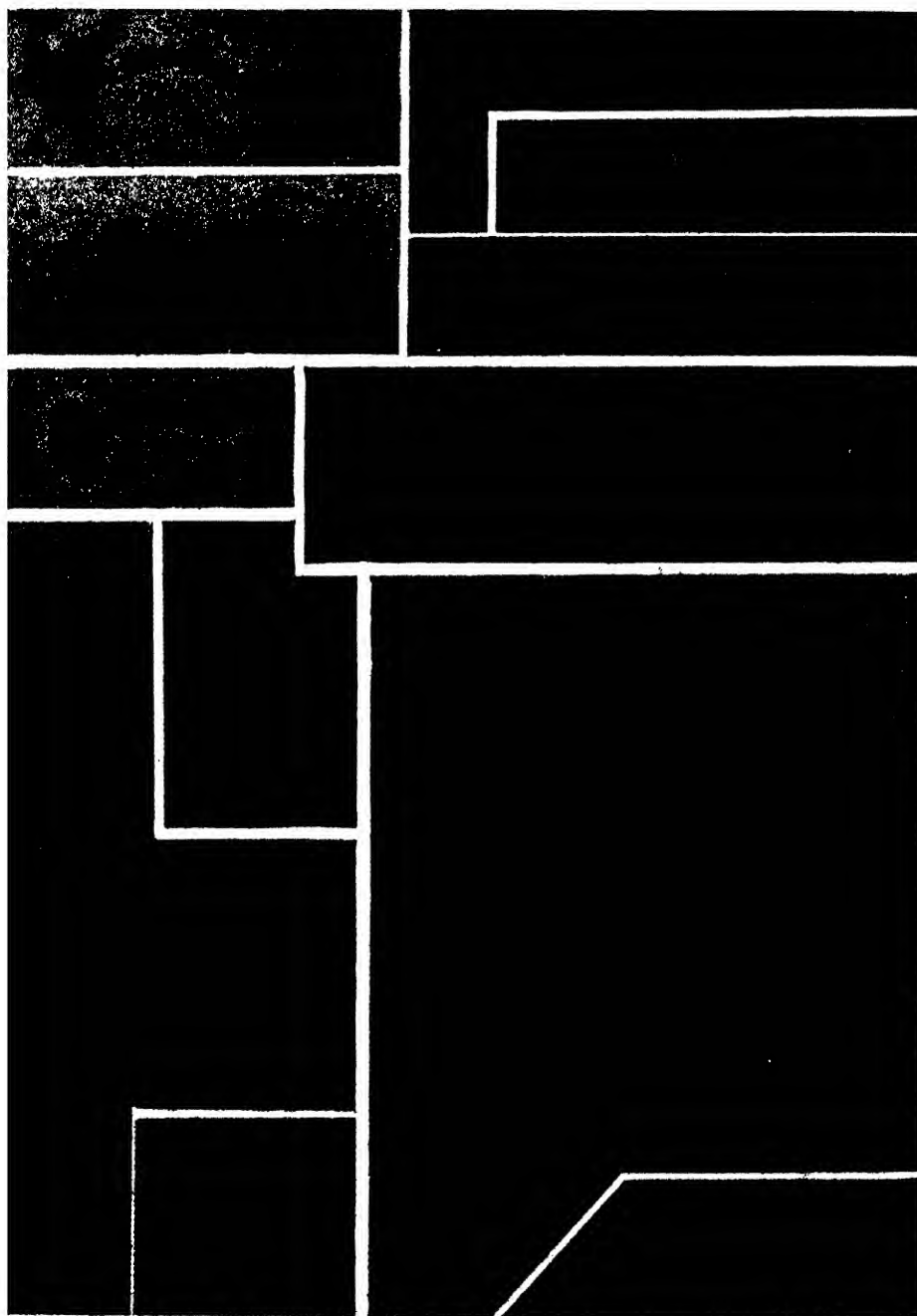
FIG. 2. Fusion bodies and hyphal network of *Puccinia graminis tritici* on nutrient-solution agar. (× 440 except where otherwise noted.)   A. Newly developed fusion body formed aerially on the tip of a germ tube, from a urediospore on the surface of the agar (out of focus).   B. Two germinated urediospores, one of which has produced a fusion body which has been retracted toward the urediospore. × 150.   C. Fusion body (arrow)

with such extensive anastomosing[1] as to form veritable networks of hyphae (Fig. 1, A). Along the strands of the hyphae were brown bodies, some almost round and others irregular in shape. The method of formation of these bodies and the networks was followed in cultures of *Puccinia graminis tritici* and *P. triticina*.

After about 5 or 6 hours' incubation the tip or lateral branch of an aerial hypha suddenly became distended to form a round body into which the contents of the germ tube were discharged (Figs. 2, A and 3, A). These bodies were about the same diameter as that of the urediospores. The suddenness with which they were formed suggested release at the hyphal tip of an internal pressure that forced the contents of the germ tube into the body. In general appearance and orange-brown color these bodies resembled those on the strands of the network (Fig. 1) except that the newly formed bodies were rounder. They are referred to herein as fusion bodies.[2]

In the usual course of events the newly formed fusion body does not remain still. After several minutes, during which the granules within it are often seen in vigorous motion, the hypha supporting it bends and the body tips downward or sideways with a slow jerking motion. If the body comes in contact with another hypha this motion is arrested and the body envelopes the contacted hypha as would a viscous drop (Figs. 2, D and 3, C). It then seems to exert a suction that gradually pulls the two hyphae taut as a stretched string. The free distal end of the contacted hypha is often suddenly pulled into the body soon after contact is made. The structure now resembles those shown in figures 2, E and 3, D. Retracted hyphae could sometimes be seen coiled within the body when the latter was broken with a micromanipulating needle soon after contact with a hypha had taken place (Fig. 3, G).

---

[1] This term is used without implication that intercommunication results from all contacts of fusion bodies with hyphae.

[2] Other types of bodies that formed on this medium were structures corresponding to appressoria and substomatal vesicles (7) and, rarely, teliospore-like bodies resembling those observed by Ezekiel (4).

---

that has been retracted toward the urediospore. D. Fusion body, produced on a germ tube from the lower urediospore, that has contacted a germ tube from the upper spore, showing dissolution of the cell wall at the point of contact. E. Fusion body (arrow) that has made contact with a hypha whose distal end has been pulled into the body. Both germ tubes have been pulled straight. F. Like E, except the distal end of the contacted hypha has not been pulled into the fusion body, *a*, but has itself produced a fusion body, *b*, either before or soon after the contact. The germ tube producing *b* is bent at right angles by the pull from the body at *a*. G. A stage following one like F, in which the fusion body formed on the contacted hypha has fallen over and contacted the germ tube that produced the first fusion body. The hyphae between the two bodies have drawn together and appear to be a single strand. H. Structure involving fusion bodies from germ tubes of five urediospores. The germ tube coming from the spore at the extreme right formed the fusion body at *a*; the germ tube from the adjacent spore formed the body at *d*; the germ tube from the spore at extreme left formed the body at *c*; germ tubes from the two lower spores produced bodies that coalesced and formed the large body at *a*. I. Network formed by fusion bodies contacting germ tubes and by subsequent coalescing and straightening of connecting hyphae. Urediospores producing the hyphae are on the surface of the agar below the network; therefore out of focus. × 300. J–L. Hyphae growing from fusion bodies. At K (arrow) is a portion of an unusually long hypha observed growing from a body that had fused with a germ tube. J and L, × 300.
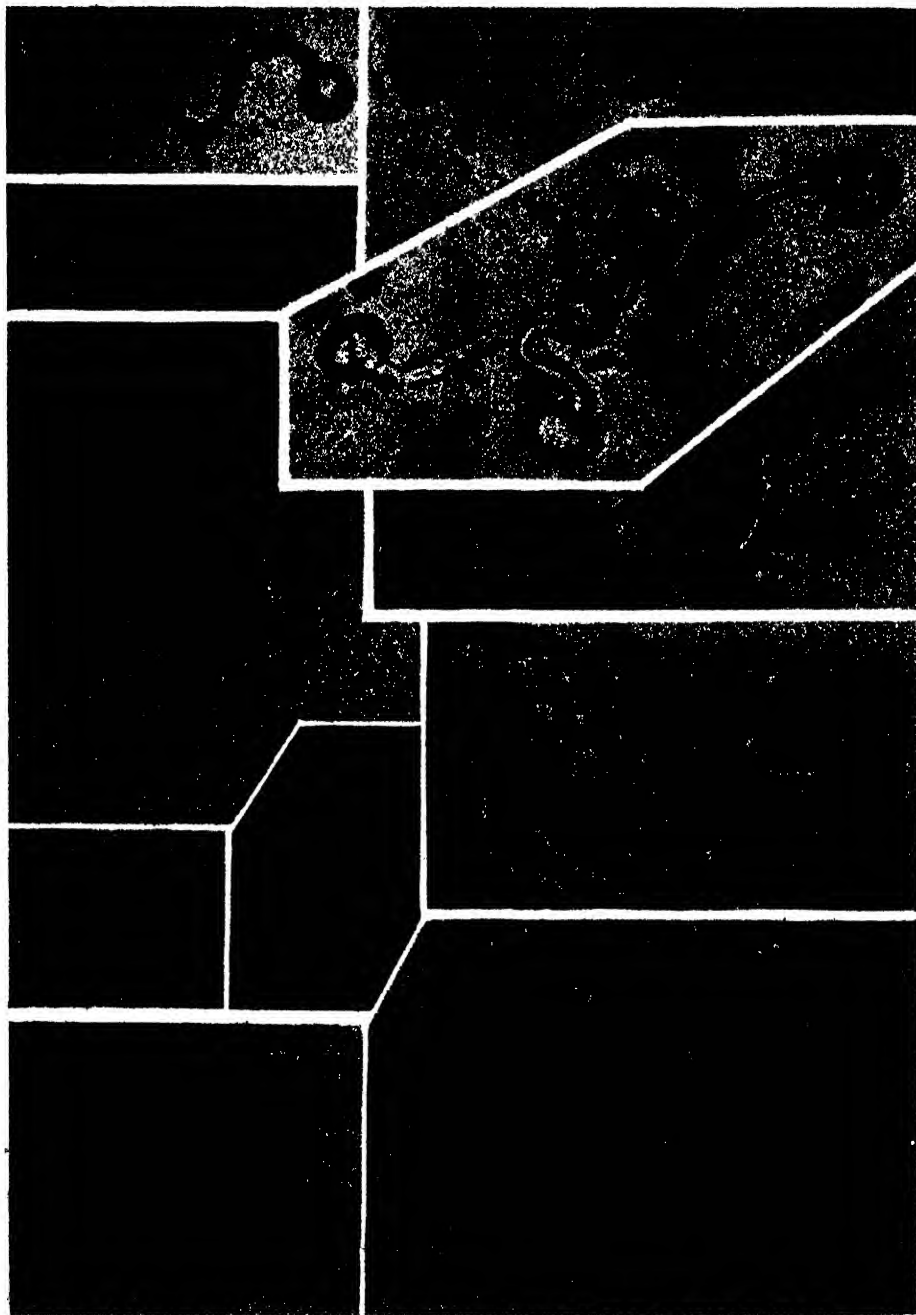
FIG. 3. Fusion bodies and hyphal network of *Puccinia triticina* on nutrient-solution agar. (× 440 except where otherwise noted.) A. Newly developed fusion body formed aerially from a urediospore on the surface of the agar, out of focus. B. A fusion body (arrow) that has been retracted toward the urediospore. C. Fusion body (arrow) formed on germ tube *a*, and fused with germ tube *b*, dissolving the hyphal wall at the point of

Sometimes the distal portion of a contacted hypha is not drawn into the body. In this case it may itself form a fusion body, as illustrated in figure 2, F, *b*, which may in turn fall on and envelop the hypha that produced the original body. The two hyphae between these bodies are pulled together and thus appear as a single strand (Fig. 2, G). When additional bodies, formed on adjacent hyphae, fall on these strands, complicated structures such as that shown in figure 2, H, are produced. This structure developed as follows: (1) the fusion body at *b*, formed on the end of a germ tube from the spore farthest to the right, contacted and fused with the germ tube from the spore farthest to the left; (2) the distal end of the latter germ tube formed a fusion body, which dropped down and fused at *c* with the germ tube from the right hand spore; (3) then a germ tube from the spore adjacent to this right hand spore formed a fusion body that contacted the strand at *d*; (4) at the other end of the strand, fusion bodies from the two lower spores contacted the germ tube from the spore to the left, and, being close together, coalesced to form the large body at *a*. Even larger bodies, formed by the coalescence of several fusion bodies, were common throughout the cultures.

Fusing of many bodies with germ tubes of adjacent hyphae may result in networks such as those illustrated in figure 1, A and in the enlargements of portions of such networks in figures 2, I, and 3, H. The very large body to the right in figure 3, H, resulted from coalescence of several fusion bodies.

Many fusion bodies fail to come in contact with hyphae. In such instances, the body appears to roll back toward the urediospore, often enveloping a considerable portion of the germ tube and becoming somewhat irregular in outline owing to inclusion of the coiled hypha (Figs. 2, B and C, and 3, B). Such was the fate of the newly formed body shown in figure 3, A. It began to retract with jerky movements just before the photograph was made. Five minutes later it suddenly engulfed the outer portion of the germ tube including the nearer branch, stopping at the level of the farther branch which remained protruding. Sometimes the body envelopes all of the germ tube and comes to rest against the urediospore.

If a newly formed fusion body touches the agar it may burst or flatten

contact. Granules were observed passing from germ tube *b* into the body. D. A fusion body (arrows) that made contact with a germ tube whose distal end was then pulled into the body and both germ tubes pulled straight. E. Two fusion bodies (arrow) that are 2 days old, therefore hardened, and three urediospores, pulled out of their normal alignment by unsuccessful attempts to pull them apart with a micromanipulating needle. The structure developed as follows: (1) the larger fusion body, formed on the germ tube coming from the spore at the right, fused with the germ tube from the spore at the left; (2) the distal end of this contacted hypha was drawn into the body, resulting in a structure like that shown in D; (3) a fusion body on the germ tube from the lower spore touched and fused with the contacted hypha to the left of the first-formed fusion body. F. A fusion body broken by contact with the agar surface before hardening. G. Fusion body (arrow) broken with a micromanipulating needle, showing a coiled germ tube in the body. H. Network formed by fusion bodies contacting germ tubes and by subsequent coalescing and straightening of connecting hyphae. The very large bodies resulted from coalescing of fusion bodies. I–J. Hyphae growing from fusion bodies. Large body at I was formed by coalescence of adjacent bodies and germ tubes as in H. × 300. K. Fusion bodies and hyphal network from germinating urediospores of *Puccinia dispersa*.

out as would a drop of liquid, and the granular contents spread on the agar, as shown in figure 3, F. In some cultures this happened to many of the bodies.

Although fluid[3] in early stages, the fusion bodies harden so that by the second day they can be manipulated with a needle without bursting. For several hours after contact is made between a body and a hypha they can be pulled apart readily with a micromanipulating needle, but after hardening has occurred the strands can no longer be easily separated. Many unsuccessful attempts were made to separate the hyphae involved in such structures, one of which, after manipulation with a needle, is shown in figure 3, E.

It appeared in several instances, such as that illustrated in figure 2, D, that there is dissolution of the wall of the germ tube at the point of contact with the fusion body. Fusion of the contents of a body with that of a contacted hypha was clearly evident in the case illustrated in figure 3, C. In this instance, observations were made over a period of 2 hours and during this time some of the granular contents of the contacted hypha, b, were seen passing into the fusion body that had formed on the germ tube a. Occasionally a few granules moved in the opposite direction but the general movement was toward the body. Further opportunity for the fusion of the contents of germ tubes occurs when two or more fusion bodies come in contact with each other and coalesce, as in figure 2, H, a, and the very large body in figure 3, H.

For these bodies to have any significance in the life history of the rusts, or in the origin of races, they should produce hyphae containing nuclei from germ tubes of different races. Therefore, a thorough search was made for evidence of growth of hyphae from the bodies.

As a rule, examination of cultures several days old showed the fusion bodies to be somewhat shrunken but otherwise unchanged. In some cultures, however, a few of them produced hyphae. Rarely bodies that had not contacted germ tubes were seen with 2 or 3 short hyphae growing from them (Figs. 2, K and L, and 3, J), while larger bodies resulting from fusion with neighboring germ tubes or with other fusion bodies sometimes had formed as many as ten hyphae. With one exception these bodies from which hyphae emerged were near mycelia of contaminants, *Rhizopus* or *Alternaria*. The exception was a body with no contaminants nearby that produced several hyphae one of which was under observation while it grew to a length of approximately 300 μ (Fig. 2, K). In a few instances a hypha of a contaminant was seen involved in a fusion body. In such cases one could not be sure but that the hyphae protruding from the body were from the contaminant, stimulated to excessive branching perhaps by the food supply provided by the mass of protoplasmic material of the fusion body. In other cases, so far as could be seen, only rust hyphae were involved. Here it would appear that production of the hyphae by the fusion bodies was stimulated by some material or condition resulting from the presence of the contaminants.

---

[3] The contents of the broken bodies were sticky and adhered to the micromanipulating needle. The mass of material that accumulated thereon was soluble in ammonium hydroxide.

The question then arose as to whether fusion bodies form on the surface of leaves. Seedlings of Little Club wheat were inoculated with urediospores of *Puccinia triticina*, race 105, according to the usual procedure, and incubated overnight. The following morning there was profuse growth of rust hyphae, and occasional networks were seen with irregular dark bodies on the strands (Fig. 1, B) similar to those formed on the agar plates (Fig. 1, A). Since fusion bodies did not form on agar except when it contained certain nutrients, there was evidently exudation of nutrient material from the plant tissue. Evidence of such exudation of substances into water drops on the surface of a leaf has been reported by Brown (3) and Arens (2).

Factors Affecting Formation of Fusion Bodies

Various factors influenced the number of fusion bodies formed. In the first place, there was considerable variability in the number of bodies formed by different spore lots of the same species germinated under what seemed to be identical conditions. In the second place, it was evident that certain environmental factors determined the number of bodies obtained with any given spore lot. Detailed studies were made, therefore, to determine the effects of nutrients, temperature, and the intensity and quality of light.
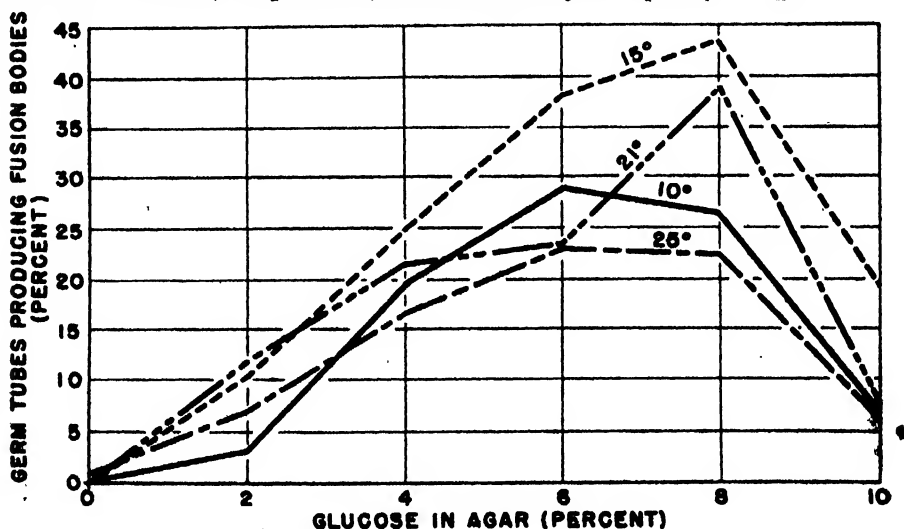
FIG. 4. Effect of glucose on the formation of fusion bodies by *Puccinia triticina* on nutrient-solution agar, incubated overnight at different temperatures (° C.).

Although good germ tube development was obtained on plain agar, almost no fusion bodies were formed unless both mineral nutrients and glucose were supplied.

Results of the first experiment to determine the number of bodies formed by *Puccinia triticina* at glucose concentrations between two and ten per cent are presented in figure 4. Percentages are based on counts of 400 germinated urediospores, except at 21° C. where 1200 were counted for each glucose concentration.

The data show that practically no bodies appeared where there was no glucose in the medium. The number increased as the glucose concentration increased, to a maximum at six to eight per cent. The number was greatly reduced at 10 per cent.

The longest germ tubes were produced where the glucose concentrations were not over 4 per cent. At concentrations of six per cent and over, reduction in hyphal development was sufficient to reduce the likelihood of a fusion body contacting a hypha, hence to reduce the extent of anastomosing. This effect is shown by the data of another experiment, presented graphically in figure 5, comprising separate counts of the bodies that contacted hyphae and
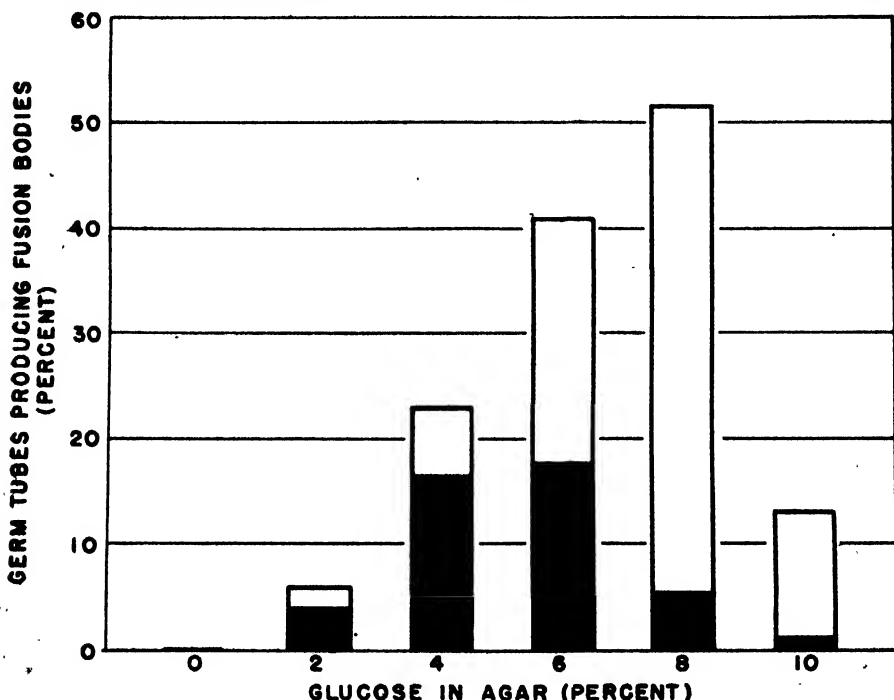
FIG. 5. Effect of glucose concentration on percentages of germ tubes producing fusion bodies. The solid parts of the bars show the percentages of germ tubes that anastomosed with neighboring germ tubes.

those that remained isolated. Urediospores of *Puccinia triticina* were germinated at 17° C. (± 1°) on agar drops suspended in Van Tiegham cells. All the drops contained the mineral constituents of the nutrient solution but differed in the concentration of glucose. Each bar of the diagram represents averages for 800 germinated urediospores, 100 counted in each of 8 cultures.

The data in figure 5 show that in the absence of glucose almost no bodies were produced. Their number increased with increasing glucose concentration up to 8 per cent, with a sharp drop in number at 10 per cent. At 2 and 4 per cent, the hyphae were long and intermingled, as when the spores are germinated on plain agar, and a relatively high proportion of the bodies con-

tacted hyphae. At the 6-per-cent level more bodies formed than at 4 per cent, but reduced growth of the germ tubes lessened the chances of contacts, so that the number of bodies fusing with hyphae was approximately the same as at 4 per cent. At the 8-per-cent level, the germ tubes were so short that a large percentage of the bodies remained isolated. At 10 per cent comparatively few bodies were formed and but a few of these made contact with other hyphae. Similar results were obtained in comparable tests with *Puccinia graminis tritici*.

As shown in figure 4, the greatest number of bodies was obtained at 15° C. at each glucose concentration except 0 and 2 per cent where relatively few bodies were formed. In other tests, however, there was very little difference over the entire range of 10° to 25° C., although usually there was a rather definite minimum at 25°. It is concluded that over the range favorable for germ tube development, temperature is not an important factor for production of fusion bodies.

Light inhibited the formation of the bodies. Urediospores of *Puccinia graminis tritici* were germinated on nutrient-solution agar in two constant-temperature rooms at 16° C., one of which was illuminated with fluorescent light having an intensity at the approximate surface of the agar of 34 footcandles,[4] while the other was kept dark. No bodies developed in the lighted room whereas the usual number formed in the dark room. Similar results were obtained when a 200-watt Mazda lamp was used as a source of illumination.

In other experiments, urediospores of *Puccinia triticina* were germinated in natural daylight on nutrient-solution agar plates placed at different distances from a north window. No bodies developed near or even at some distance away from the window where the light intensities were 100 and 20 foot-candles, respectively. A few bodies were formed where the intensity was one foot-candle. Only in very dim light, 0.4 of a foot-candle, were there as many bodies as in complete darkness.

To determine the effect of light quality on fusion-body formation, germinating urediospores of *Puccinia triticina* were exposed to light from a 200-watt Mazda lamp transmitted through red, yellow, and blue Wratten filters, respectively. The intensity of the light near the surface of the agar was approximately 45 foot-candles behind the red and yellow filters and 10 foot-candles behind the blue one. An additional spore lot was germinated in darkness.

The data in table 1 and similar results obtained with *Puccinia graminis tritici* and *P. coronata avenae* showed that the components of white light responsible for the inhibition of fusion bodies are the shorter wave lengths at the blue end of the spectrum. Since the bodies formed normally behind the yellow filter that transmitted down to about 5000 Å, it can be concluded that the inhibiting wave lengths are those between 4000 and 5000 Å. It should be noted that the intensity of the blue light that produced the inhibit-

[4] These intensity measurements are only approximate because the Weston Illumination Meter used was standardized for sunlight.

ing effect was but a fraction of that of the red and yellow light that had no effect on body formation.

It was noted in the course of these investigations that the germ tubes grew in a direction away from the source of white light, as has been observed by others (5, 6, 8, 11). The data in table 1 show that the blue constituents of white light are responsible for this orientation, as found previously by Forbes (5). The germ tubes subjected to the light transmitted by the yellow and red filters were not oriented although this light was much stronger than that behind the blue filter.

An impression was obtained from the various experiments that the most fusion bodies were formed by relatively fresh spores. To determine the effect of age of spores, counts were made every two weeks on a spore lot of *Puccinia graminis tritici* and one of *P. triticina* of the same age stored in the refrigerator at 13° C. The spores were germinated on nutrient-solution

TABLE 1.—*Effect of red, yellow, and blue light on fusion-body formation and orientation of germ tubes in Puccinia triticina germinated on nutrient-solution agar*

	Color and intensity of light			Fusion bodies	Orientation of germ tubes
		Transmission in the visible spectrum			
Filter no.	Color	Wave lengths in Angstrom units	Intensity in foot-candles[a]		
.. ..	Darkness	....	0	Many	0
2412	Red	6000–7200	45	Many	0
3486	Yellow	5000–7200	44	Many	0
5543	Blue	4000–5400	10	0	+

[a] Measurements are approximate because the Weston Illumination Meter used was standardized for sunlight.

agar containing 6 per cent glucose, and incubated overnight at 13° C. For the first three months there was no significant change in the percentages of germ tubes that produced fusion bodies. The percentages ranged from 24 to 41 for stem rust and 26 to 42 for leaf rust. After three months the figures decreased, somewhat more rapidly for stem rust than for leaf rust, until after about four and a half months the percentages were all less than 10.

There was little difference between the various species of rust with respect to the number of fusion bodies formed, with the exception of *Puccinia sorghi* whose germ tubes showed such a strong tendency to grow down into the agar that relatively few of them produced bodies.

DISCUSSION

Reports of fusions of hyphae of rust fungi within the host plant have been made by Plowright (10, p. 5) and Allen (1). The former stated that mycelia ramifying within the tissues united to form an "anastomosing irregular network"; and the latter found that intercellular hyphae of *Puccinia triticina* sometimes fused, forming an "enlarged mass" at the point of union,

from which grew other hyphae. The present paper, based on work with six species of cereal rusts, presents evidence of fusion by means of special bodies on germ tubes from urediospores germinated on a nutrient-containing agar.

Fusion bodies may have been seen by Plowright (9 and 10, p. 32) on germ tubes of *Puccinia graminis* [*Uredo linearis*] from urediospores germinated on a drop of water. In describing them he said the terminal extremity of the tube became expanded in a globular manner, "into which all the yellow endochrome accumulated." He concluded that they were "endochrome reservoirs" (9) or "spores of reserve" and implied that they formed on the surface of leaves (10, p. 32).[5]

No other report of these round bodies has been found in the literature. Apical swellings were observed by Ezekiel (4) on various media and were described by him as teliospore-like. Bodies resembling them were occasionally seen in the present investigation. They were quite unlike the spherical fusion bodies.

Some networks of hyphae, with fusion bodies on the strands, were observed on the surface of wheat leaves inoculated with *Puccinia triticina*. Since a supply of mineral nutrients and glucose was essential for the development of the bodies on agar, it is assumed there was exudation of nutrient materials from the inoculated leaf tissue.

Hyphae from fusion bodies developed occasionally in agar-plate cultures of both *Puccinia triticina* and *P. graminis tritici*. The nuclear condition of these hyphae remains to be determined; and proof of the practical significance of the fusion process will depend on the results of pathogenicity studies. The observations made thus far suggest strongly that here may be a method whereby new races may arise in the absence of an alternate host. The process may account for the numerous races of such rusts as *P. triticina* in regions where the aecial stage is rarely, if ever, found.

SUMMARY

Germ tubes from urediospores of cereal rusts (*Puccinia graminis tritici*, *P. triticina, P. dispersa, P. coronata avenae, P. hordei, P. sorghi*) grown on agar containing mineral nutrients and glucose produced bodies that fused with neighboring germ tubes, forming anastomosed networks of hyphae above the level of the agar surface.

The bodies, designated "fusion bodies," occasionally produced several short hyphae. These hyphae formed on isolated bodies as well as on those that had fused with neighboring germ tubes. Such hyphae were seen in older cultures contaminated by *Rhizopus* and *Alternaria*, by-products from which may have had some stimulating effect on their production.

Both mineral nutrients and glucose were essential for normal production of the fusion bodies. They formed in greatest numbers on media containing 6 to 8 per cent glucose but the best concentrations for network formation

---

[5] Plowright refers here to work (9) in which he apparently germinated the spores on water drops, leaving doubt as to whether he actually saw the bodies on leaves.

were between 4 and 6 per cent, because of more extensive development of hyphae at these concentrations.

Fusion bodies formed at all temperatures favorable for hyphal development. They were inhibited by daylight and by artificial illumination from Mazda and fluorescent lights. This effect of light was traced to the short wave lengths at the blue end of the spectrum, between 4000 and 5000 Ångstrom units.

Hyphal networks with fusion bodies on the strands occasionally were found on wheat leaves inoculated with urediospores of *Puccinia triticina*.

PLANT INDUSTRY STATION,
    BELTSVILLE, MARYLAND.

LITERATURE CITED

1. ALLEN, RUTH F. A cytological study of *Puccinia triticina* physiologic form 11 on Little Club wheat. Jour. Agr. Res. [U.S.] 33: 201–222. 1926.
2. ARENS, K. Die kutikulare Exkretion des Laubblattes. Jahrb. Wiss. Bot. 80: 284–300. 1934.
3. BROWN, W. Studies on the physiology of parasitism. VIII. On the exosmosis of nutrient substances from the host tissue into the infection drop. Ann. Bot. 36: 101–119. 1922.
4. EZEKIEL, W. N. Studies on the nature of physiologic resistance to *Puccinia graminis tritici*. Minn. Agr. Exp. Sta. Tech. Bul. 67. 1930.
5. FORBES, I. L. Factors affecting the development of *Puccinia coronata* in Louisiana. Phytopath. 29: 659–684. 1939.
6. FROMME, F. D. Negative heliotropism of the urediniospore germ-tubes of *Puccinia rhamni*. Amer. Jour. Bot. 2: 82–85. 1915.
7. HURD-KARRER, ANNIE M., and H. A. RODENHISER. Structures corresponding to appressoria and substomatal vesicles produced on nutrient-solution agar by cereal rusts. Amer. Jour. Bot. 34: 377–384. 1947.
8. MAINS, E. B. Some factors concerned in the germination of rust spores. Rpt. Mich. Acad. Sci. 17: 136–140. 1916.
9. PLOWRIGHT, C. B. Some observations on the germination of the Uredines. Grevillea 10(no. 56): 136–142. 1882.
10. ————. A monograph of the British Uredineae and Ustilagineae. London, 1889.
11. STOCK, F. Untersuchungen über Keimung und Keimschlauchwachstum der Uredosporen einiger Getreideroste. Phytopath. Ztschr. 3: 231–279. 1931.

BURN BLIGHT OF JACK AND RED PINE FOLLOWING SPITTLE INSECT ATTACK[1]

R. H. GRUENHAGEN, A. J. RIKER, AND
C. AUDREY RICHARDS[2,3]

INTRODUCTION

A severe epidemic of burn blight appeared recently in Wisconsin and Michigan on jack and red pine. In 1940 MacAloney and Secrest (6) noted pines dying in lower Michigan. In 1941 dying red pines were found in northern Wisconsin. The disease was found in 1942 on both jack and red pine in Wisconsin, in Forest and Marinette Counties, respectively. Cortical browning and fungus fruit bodies were observed on killed tissue follow-

TABLE 1.—*Number of areas and approximate acreages in Wisconsin showing the burn blight disease on jack or red pine, 1945*

Counties with infection	Date of first report	Areas infected	Approximate acres involved
		Number	*Number*
Florence	1943	6	640
Forest	1941	3	120
Iron	1945	1	10
Langlade	1944	5	40
Marinette	1941	28	2600
Oconto	1943	8	2100
Oneida	1943	4	160
Price	1945	1	....
Vilas	1943	8	780
Total	....	64	6450

ing attack by the spittle insect (*Aphrophora saratogensis* Fitch) on these two species, viz., *Pinus banksiana* Lamb. and *P. resinosa* Ait. The disease has not been reported on other tree species.

The distribution of burn blight in Wisconsin in 1945 involved approximately 6500 acres scattered through 64 areas in 9 northern counties, as indicated in table 1. It has appeared also in the Manistee Forest in lower

Michigan and in several areas in upper Michigan. Similar heavy damage to the jack and red pine has been reported in the Cass Lake region of Minnesota.

The disease is known to be distributed over a much smaller area than is the associated spittle insect, which has been reported from Nova Scotia south to Florida, west through the Lake States and Ontario to British Columbia and California (1).

Wherever it has appeared burn blight has caused alarm. For example, the commercial lumber interests have wondered whether they will ever have the local red pine on which they were counting. The pulp and paper industries have seen ruined some of their prospects of local jack pine. The foresters have watched the dying of young stands and have questioned what can be done with the areas selected for growing these two species.

<center>SYMPTOMS</center>

From a distance a heavily diseased stand appears as if a fire had singed the tree tops, turning them brown and leaving the lower branches relatively green for a time. This has suggested the name "burn blight." A typically diseased tree is shown in figure 1, A. Closer examination revealed that although the tops died first, there were from few to many "flags" throughout the rest of the tree and many spittle insect punctures on living twigs.

The sequence of symptoms in a typical case on jack pine was as follows. Before any foliage discoloration appeared, insect punctures were visible at the cambium when the bark was peeled away (Fig. 1, C, I, and J). The insect feeding punctures shown in figure 1, C were tiny, light tan flecks, sometimes surrounded by infected spots that were dark, somewhat watersoaked, and coalesced as they enlarged. The subsequent spreading necrosis shown in figure 1, D, E, and H was a typical sign of fungus invasion associated with yellowing and browning of the twigs. The necrosis soon completely girdled the branch and advanced both up and down. When the fungus was growing rapidly the needles often turned brown next to the twig before they died at the outer end. The usual pattern was for the disease to start in the twigs in the upper part of the tree and to work toward and down the main stem. Occasionally the necrotic margin passed noninfected branch whorls. The twigs on these whorls then changed from green to yellow and brown. The disease has been observed at times to progress from affected side branches, which it killed, to the main stem and then to spread up and down through the bark and cambium from that point.

These symptoms have been attributed to desiccation both because of the removal of liquid by spittle insects and because of the resin blocks in the wood. Since the symptoms of burn blight and desiccation needed clarification, the progressive appearances of twigs dying from lack of moisture were examined.

Portions of jack pine trees were dried with varying degrees of rapidity. Branches removed from the tree and dried rapidly in the laboratory showed
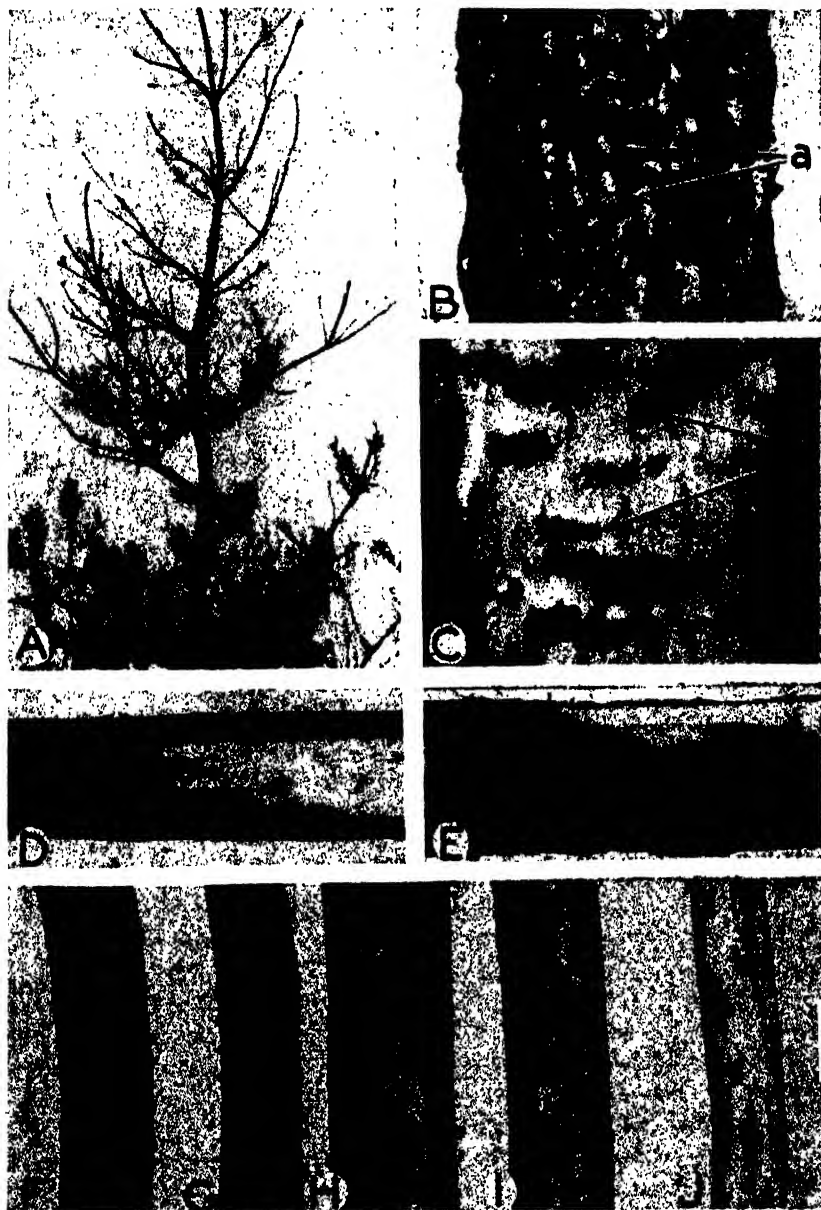
FIG. 1. A. Burn blight has killed the top of this 10-foot jack pine. B. *Chilonectria* fruiting (a). C. Brown necrotic tissue (b) surrounding spittle insect feeding punctures (red pine). D. Relatively sharp margin between dead and healthy under-bark tissue. E. Coalescing fungus-invaded areas. F. Branch killed by desiccation is tan without sharp margin. G. Branch killed by *Chilonectria* is chocolate brown. H. Advanced necrotic margins. I and J. Insect injury in cambial region and in wood, respectively.

little change in the color of the cortical tissues. Branches injured on the tree by cuts of varying depths and dried slowly developed a tan and dry cortex with no sharp margin between the living and dead tissues (Fig. 1, F). Symptoms of desiccation on trees injured by cuts on the main stem corresponded with those for cut branches on the tree. In no case were fungus fruit bodies observed on tissues in these experiments. Such dried material was easily differentiated from the chocolate-brown and moist tissues newly invaded by the fungus (Fig. 1, G), tissues which had characteristic sharp margins between infected and healthy portions (Fig. 1, H) and on which reddish brown perithecia soon developed (Fig. 1, B, a).

Small orange to brown, erumpent perithecia appeared on diseased twigs, branches, and stems in as short a time as 4 weeks after infection. They were readily visible on jack pine (Fig. 1, B, a), but were frequently obscured by the bark scales on red pine.

The disease symptoms on red pine were similar but varied in two important ways: (1) Whenever jack and red pine were found growing together the incidence of insect punctures was higher on the red pine. (2) In spite of this, the disease progressed more slowly on the red pine. These two facts suggested that in the mixed stands red pine was more tasteful to the spittle insects than the jack pine and that the red pine was more resistant to the fungus. After heavy infestations of Saratoga spittle insect, red pines have been observed to recover more frequently than jack pines.

Diseased jack pine branches were sectioned and stained with Pianeze III, B. The fungus had a prolific, much-branched mycelium that invaded the cortex, killed the cambium, and penetrated to a depth of 8 or 9 cells into the xylem. The fungus frequently killed the twigs or stems by girdling them.

The twigs were killed first, perhaps because they were more frequently punctured by the insects and more rapidly girdled by coalescing fungus lesions than large branches. However, on some 12-foot trees the bark and cambium were invaded progressively down to the ground.

CAUSAL AGENT

The fungus fruiting on jack pine was identified by M. P. Backus as *Chilonectria cucurbitula* (Curr.) Säcc. Although from external appearance this organism resembles a true *Nectria*, the formation of secondary ascospores places it in the genus *Chilonectria*. These spores are small and often so numerous in the ascus that they appear as a granular mass which is easily misinterpreted. Descriptions are given by Saccardo (7) and Ellis and Everhart (2). So far as the writers know, this is the first time *C. cucurbitula* has been associated with a serious disease; therefore, its pathogenicity was examined critically.

Pathogenicity

The association of *Chilonectria* with this disease was first noted in the fall of 1943 when typical fruiting bodies occurred on almost every killed

branch.   Fruiting bodies were found in 1945 on approximately 1000 affected branches.

In the fall of 1943 cultures were made from more than 40 diseased pine twigs.   *Chilonectria* predominated in cultures made from the advancing brown margin in the cambium and cortex, and *Pullularia pullulans* DeB. appeared commonly from the bluish-black stains in the vascular tissue.

The culture technique employed in 1944 and 1945 was adapted from that used by Henry et al (4).   The twig or branch was stripped of needles and was dipped momentarily in 95 per cent alcohol.   The excess alcohol was shaken off and the remainder burned away.   The outer bark was removed with sterile scalpels and about 10 chips were cut from the region of most recent discoloration and were transferred aseptically to 2 per cent malt agar in Petri dishes.   After 3 days at room temperature the fungus commonly appeared as a light-colored, delicate mycelial growth, closely appressed to the agar, and radiating from the chip for a distance of about 3 to 5 mm. After 6 days the growth was more dense near the chip but was still delicate at the margin.   By that time a series of concentric zones was apparent. After 7 to 10 days the surface of the growth was covered by hyaline microconidia which were orange in mass.   The mycelium seldom became fluffy, and then only at high temperatures (32° to 36° C.).   Final readings were made after about 7 to 10 days on all the chips.   The growth on agar was characteristic and not easily confused with common contaminants.

In plantings made from the margin of cortical lesions, *Chilonectria cucurbitula* grew from all 49 jack pines and 7 red pines cultured in 1944 and from the 80 jack pines and 27 red pines cultured in 1945.

Plantings were also made from 40 healthy jack pines and 20 healthy red pines.   *Chilonectria cucurbitula* was not isolated from any of these healthy trees.   Among the 700 necrotic areas[*] around insect punctures observed in 1944 and 1945, 6 and 13 per cent, respectively, were infected. Evidence is presented later that the insects carried the fungus, at least externally.

Several inoculation procedures were followed during 1944 and 1945, in which a number of types of wounds were employed.   Inoculations attempted without wounds all gave negative results.   The wounds effective in providing a portal of entry were: needle punctures with a side-flow hypodermic needle (5), multiple punctures by an inoculator made with a large number of entomology mounting pins stuck close together through a piece of soft wood, and bruises made with a small hammer as used with *Hypoxylon* (3). Spore suspensions in distilled water were commonly sprayed on the two latter types of wounds.   However, in some cases the punctures were made under spore suspensions.   The multiple punctures resembled more closely the inoculations associated with the insect injuries and were used most frequently.   With a large number of punctures the necrotic spots coalesced more rapidly with a correspondingly reduced time for girdling and flagging.

[*] The cambium around a spittle insect puncture showed a slight discoloration often 1 or 2 mm. across, in which the cells appeared dead.

Spores for the inoculations came either from recently killed jack pine branches or from cultures from infected jack pine tissue. Spore suspensions for inocula were prepared from three sources: (1) For natural spore material, twigs with abundant fruiting bodies were immersed about a day in distilled water. The twigs were removed and the spore suspension containing a mixture of ascospores and conidia was poured through cheesecloth to remove the debris. The resulting spore suspension was about 99 per cent *Chilonectria*. (2) Isolations from the advanced necrotic margins yielded two isolates which were purified by monoconidial transfers and were used as stock cultures. (3) Fruiting bodies on jack pine were crushed in sterile water and eight single ascospores were picked and grown as stock cultures. In the last two cases the cultures formed orange to pink colored masses of conidia, which were suspended in water for the inoculations.

The results of inoculations, showing the repeated fulfilling of Koch's postulates, are summarized in table 2.

The symptoms that followed inoculation of red and jack pines corresponded closely with those in nature.

The results from the various inoculations may be summarized as follows: At Phelps, Wisconsin, only 3 of the 120 control trees receiving only punctures developed any infection. This would doubtless have been higher if the tests had been conducted in an area where the fungus spores were abundant. No external disease symptoms were noted on the 10 trees inoculated with *Pullularia pullulans*, although this fungus stained the wood. Of the 250 trees inoculated with *Chilonectria*, 248 developed typical cortical symptoms. Of 110 left long enough, every one developed typical flagging. *Chilonectria* was reisolated from 108 of these trees.

In the greenhouse at Madison, Wisconsin, 60 jack pine trees receiving only punctures developed no disease symptoms. Among the 120 trees inoculated through punctures with *Chilonectria*, 116 developed cortical symptoms and flagging, and *Chilonectria* was reisolated from all.

In field plots at Madison, where jack pines were growing vigorously on good soil, but where there was neither spittle insect nor *Chilonectria*, 90 twigs punctured but not inoculated developed no *Chilonectria* lesions. Of 58 such twigs inoculated with *C. cucurbitula*, cortical symptoms and flagging developed on 46. The fungus was reisolated from 37 of the 46. The fungus had little tendency to spread down the twigs from the inoculation punctures, probably because of tree vigor.

The disease developed in 410 cases of the 428 inoculations made. In 261 cases out of 272 trials in which *Chilonectria* was inoculated into jack pine twigs, typical symptoms developed and the causal fungus was recovered.

Although *Chilonectria* thus appears as a pathogen, which may operate independently through wounds, the spittle insect seems important for natural development of burn blight.

TABLE 2.—*Summary of inoculations in 1944 and 1945 of jack pine with spores of Chiloneotria cucurbitula*

| Plot location, treatment, and origin of spores | Trees treated | Date of treatment | Trees with: | | Chilo-neotria reiso-lated |
			Disease in the fall	Flagging the next summer	
	No.		*Per cent*	*Per cent*	*Per cent*
Phelps, Wis. (in woods)					
Punctured, not inoculated	20	Aug. 23, 1944	0	0	0
Do	40	June 19, 1945	2	0	2
Do	20	June 20, 1945	0	0	0
Do	10	June 25, 1945	0	0	0
Do	10	June 29, 1945	0	0	0
Do	20	Aug. 17, 1945	0	0	0
Punctured, inoculated with spores from:					
Perithecia	20	Aug. 23, 1944	100	100	100
Lesion culture No. 1	20	Aug. 23, 1944	100	100	100
Do No. 2	40	June 19, 1945	100	100	100
Do No. 2	10	June 20, 1945	100	100	100
Do No. 2	10	June 25, 1945	100	100	100
Do No. 2	10	Aug. 17, 1945	80	100	80
Do No. 3	60	June 29, 1945	100	42	..
Single-spore culture No. 1	10	Aug. 17, 1945	100	20	...
Do No. 2	10	Aug. 17, 1945	100	50	...
Do No. 3	10	Aug. 17, 1945	100	0	...
Do No. 4	10	Aug. 17, 1945	100	30	...
Do No. 5	10	Aug. 17, 1945	100	80	...
Do No. 6	10	Aug. 17, 1945	100	40	...
Do No. 7	10	Aug. 17, 1945	100	80	...
Do No. 8	10	Aug. 17, 1945	100	60	...
			Disease 6 weeks after inoculation	Flagging 10 weeks after inoculation	
Madison, Wis. (in greenhouse)[b]					
Punctured, not inoculated	60	Feb. 10, 1945	0	0	0
Punctured, inoculated with spores from:					
Lesion culture No. 4	60	Feb. 10, 1945	98	98	100
Do No. 5	60	Feb. 10, 1945	95	95	100
Madison, Wis. (in nursery)					
Punctured, not inoculated	90	June 15 to July 17, 1945	0	0	0
Punctured, inoculated with spores from perithecia	58	June 15 to July 17, 1945	79	79	80

[a] The cortical symptoms were characteristic and no reisolations were attempted.

[b] A parallel series was run on 180 red pines with 100 and 97 per cent showing disease, respectively, for culture Nos. 4 and 5. *Chiloneotria* was recovered from every diseased seedling.

RELATION OF THE SPITTLE INSECT TO THE DISEASE

The Saratoga spittle insect, which is associated with the disease, has been found in the Lake States for many years. However, it was not reported as causing damage in pine stands until 1940 when MacAloney and Secrest (6) and Secrest (8) observed injury in lower Michigan.

The association of the spittle insect with burn blight was apparent not only from the frequent presence of the adult insects on diseased trees, but also from an examination of feeding punctures on several thousand trees throughout the various areas involved. Feeding by this insect preceded the discoloration of the twigs. However, death of the twigs often occurred only after 8 to 10 months. Occasionally, however, browned areas infected with *Chilonectria* were found surrounding holes made by other insects, *e.g.*, pitch mass borers, or developing near wounds made by deer, rabbits, or other agencies.

In the Phelps area the nymphal and instar stages of the spittle insect developed at the crown of various low-growing plants during May, June, and early July in 1944 and 1945. The adults appeared in early July and were active through July, August, and September. Their feeding on pine was heaviest on 1-year-old twigs and progressively less on current-year twigs, or on 2-, 3-, and 4-year-old twigs. There was little if any feeding upon branches or stems over 8 years old or over 1 inch in diameter. The fungus, however, spread into branches and stems from 1 to 14 years old.

Enormous numbers of spores of *Chilonectria* exuded whenever the mature perithecia were moistened, so spores were common on the foliage of the jack pine where they might be picked up by crawling insects.

Various stages of 85 spittle insects were collected and each was touched lightly about 20 times to 20 ml. sterile 2 per cent malt agar in Petri dishes. No fungus developed from the nymphs, and from only one of the fourth and fifth instars. However, *Chilonectria* was obtained from all bodies and mouth parts of adults used in 1944 and 1945. The obvious association of the fungus with the adult insects suggested further work on them as possible vectors.

Necrotic areas surrounding 400 insect punctures were examined in jack pine specimens from three northern Wisconsin regions. The total number of necrotic areas surrounding insect punctures and the number of such areas that contained the fungus were counted and recorded as shown in figure 2. Except in recent infections, the infected areas were larger and darker than noninfected discolored areas around punctures. Here a correlation appears between the number of necrotic areas per square centimeter and the number of such areas that were infected. The ratio of infections per square centimeter increased with an increase in the number of feeding punctures per square centimeter, as shown by the upward trends of the curves. The ratio of infected spots per square centimeter to number of necrotic areas was greater in 1945 than in 1944, possibly owing to the larger amount of fungus inoculum available as the epidemic increased.

Experiments to determine the relative parts played by the fungus and by the insects in the initiation of the disease were conducted in 1945 near Phelps, Wisconsin, on a 20-tree jack pine plot with 5 trees in each treatment. The trees were from 4 to 5 feet high and were growing vigorously in an area well removed from heavily infected trees. The trees were punctured

at the rate of 15 punctures per square centimeter along the top 2 feet of
the main stem and the associated side branches. Sleeve-type wire cages 8
inches in diameter and 2 feet long with attached cloth sleeve ends were used
to enclose the upper parts of the trees.

Fifteen puncture wounds per square centimeter did not produce the
disease symptoms. The fungus introduced by spore suspension or by
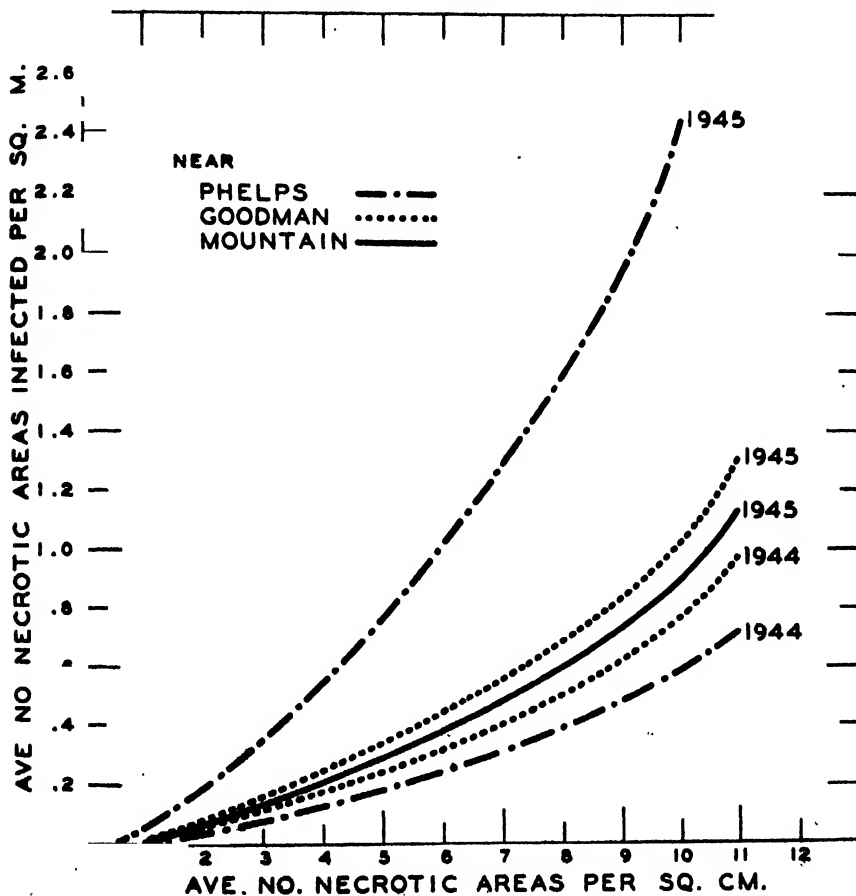
FIG. 2. Relation between the number of insect-initiated and necrotic cortical areas
per square centimeter and the number of such areas containing *Chiloneotria*.

insects did produce disease symptoms. A combination of heavy fungus
infection introduced by spores and insects killed the trees rapidly.

In these tests attempts to determine how many insects were necessary to
kill twigs by their feeding alone were unsuccessful, because the fungus could
not be excluded.

TREE VIGOR AND BURN BLIGHT DEVELOPMENT

The effect of tree vigor on disease development was studied by weakening
vigorously growing trees of similar size in various ways. In a trial begun

on August 3, 1944, the soil was removed and about one-half of the roots
were cut from 5 trees.   All dead twigs and branches were removed from
these trees and from 5 nontreated controls.   About 1 year later, the non-
treated controls had a total of 49 inches of dead twig length caused by burn
blight, an average of approximately 10 inches per tree.   The root-pruned
series, however, had a total of 198 inches of such dead twigs and branches,
an average of approximately 40 inches per tree.   This indicated that root-
pruned trees were more susceptible to fungus attack.

Further evidence was secured from vigorous trees on which mechanical
punctures were made into 45 uniform-sized branches.   The needles were
removed by hand from the 1-year-old wood and the area of the bark was

TABLE 3.—*Summary of puncture test on jack pine, July 12, 1945, to September 20,
1945, near Phelps, Wisconsin*

Mechanical punctures per square centimeter	Average diameter of necrotic areas[a]	Condition of twigs
No.	*Mm.*	
0	0	Foliage green and branches healthy.
5	1	Foliage green, small under-bark spots, not infected.
10	1 to 2	Foliage green, small under-bark spots, not infected.
15	2 to 3	Foliage green, small under-bark spots, 0.1 per cent of spots infected.
20	3 to 5	Foliage green, 5 per cent of spots infected.
25	3 to 10	Foliage green, about 50 per cent of the cortical area with infected spots coalescing and drying.
30	5 to 10	Foliage yellow, 50 to 75 per cent of cortical area infected and drying.
35	Girdled	Foliage yellow, 75 to 90 per cent of cortical area covered by coalescing infected areas. One of 5 branches dead.
40	Girdled	Two of 5 branches were dead with the fungus fruiting and 3 branches had 90 per cent of the cortex infected with coalescing spots. Three-quarters of the foliage was dead.

[a] No spores were artificially applied.   The average is that from all 5 treated branches.

calculated.   From 5 to 40 punctures per square centimeter were made with
the multiple-pin block.   Each treatment plus an unwounded control was
replicated 5 times.   The trial ran from July 12 to September 20, 1945.
(See table 3.)   The punctures were not inoculated artificially.   However,
Chilonectria was isolated from all branches in which the necrotic spots
coalesced.   From these results it appears that natural infection takes place
through punctures and that widely scattered punctures are not so likely to
become infected as those placed close together.   This corresponds to the
upturn in the curves of figure 2 showing more infections with an increase
in number of insect-induced necrotic areas per square centimeter.   This
evidence, along with that from root-pruned trees, suggests that injury may
increase susceptibility to natural infection by *Chilonectria*.

Since killing many twigs greatly reduced the functional needle-bearing
area of a tree, the question arose as to whether this increased susceptibility

to fungus invasion. Some trials of drastic pruning were made during the summer of 1944. In one series with 10 replications approximately 0, 25, 50, 75, and 90 per cent of the needle-bearing areas were cut away. In another series with suitable controls and replicated 10 times in 4 locations in Wisconsin, about 80 per cent of the needle-bearing areas were cut off. By November, 1945, the severe pruning apparently had no effect upon natural disease development. If anything, the pruned trees showed less disease development than did the unpruned, although the difference was not statistically significant. All the trees, except one with 90 per cent of the foliage removed, survived. The new growth on the pruned trees was particularly vigorous.

The effect of various vigor-reducing treatments upon the development of disease symptoms following artificial inoculation was examined. The treatments were (1) none, (2) removal of all branches from the lower half of the tree, reducing the needle area by 60 to 80 per cent, (3) partial girdling of the main stem below the point of inoculation (last 3 inches of 1943 growth and first 3 inches of 1944 growth), and (4) root pruning to remove about 50 per cent of the roots. These trees were then inoculated in the current-year wood of the leaders with the hypodermic needle inoculator as follows: (1) control, distilled water; (2) a suspension of *Chilonectria* spores from the single-spore isolate No. 1; and (3) a suspension of *Chilonectria* spores from field-collected fruiting bodies. These treatments were replicated 5 times under each of the 4 types of pre-inoculation treatments. The series was established on August 23, 1944, in the Phelps, Wisconsin, area.

The inoculated trees turned yellow or brown above the point of inoculation and there was considerable cortical necrosis. By the next spring all the inoculated trees had dead tops above the point of inoculation. The tops of the control trees were still green and healthy.

Detailed examination of the trees was made during September, 1945, in the laboratory. Reisolations were attempted from every tree. The fungus was not isolated from the control trees and was recovered from all inoculated trees.

Spread of the fungus in infected trees was also studied. The cortex in the diseased region was removed and the advance down the stem from the lowest needle puncture was measured and the results averaged for treatments and for source of inoculum. The averages are presented in figure 3. Although the fungus advanced to some extent on vigorous trees, it moved considerably farther on weakened trees. This provides further evidence that tree vigor influences burn blight development, and suggests that site factors might influence susceptibility.

Some indications of the effect of site quality were observed on two plots, one poor and the other good. But no effort was made to isolate the effect of each factor, such as soil type and fertility, drainage, exposure, moisture, slope, ground cover, overstory, and others. The study areas were both on

a light sandy loam soil and were only about ¾ mile apart. On the poor site
the soil moisture content (dry weight basis) at a 6-inch depth was 6.1 per
cent at a time when it was 11.7 per cent on the good site. The soil reaction
in both plots was pH 5.5. The average length of 25 growing tips recorded
on June 15, 1945, was 2.3 inches for the poor site and 4.1 inches for the
good site.

Disease development was observed on these plots from June, 1944, to
November, 1945. The records showed that there was little top dying on the
good site while all 25 trees on the poor site had died down from the top at
least one-fourth the length of the main stem. The total length of dead twigs
and branches was measured and the records showed the dead lengths to be
3 feet on the good site and 423 feet on the poor site. Because the insects
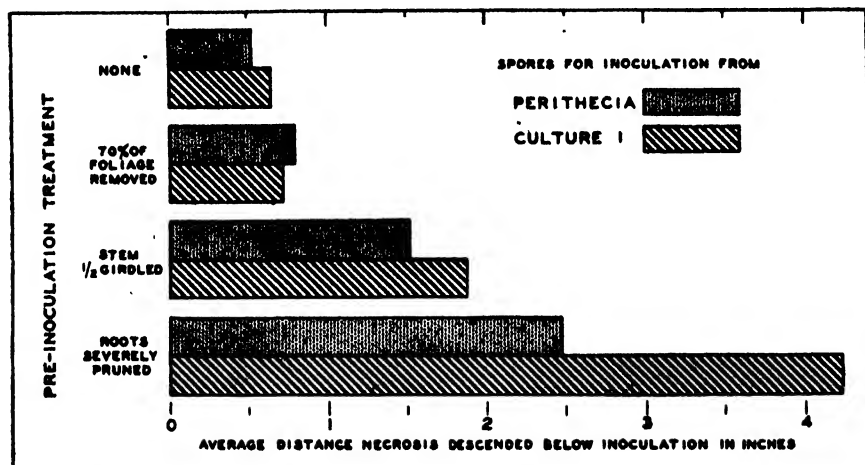
FIG. 3. Relation of pre-inoculation weakening (average for 5 trees) to the rate
of fungus advance down the main stem of jack pine.

may have inoculated the trees on the poor site more than those on the good
site, the reaction to artificial inoculations was examined.

Puncture inoculations were made with a spore suspension from single-
spore culture No. 1 on 5 jack pine stems in each plot. The records taken
14 months later, in September, 1945, showed that the top of every tree had
been killed in each area. However, on the good site the fungus had ad-
vanced down the stem an average of only 1.3 cm. below the lowest inocula-
tion wound, while on the poor site the advance averaged 10.9 cm. These
observations suggested that the site and the resulting condition of the trees
influenced the natural incidence as well as the artificially induced develop-
ment of disease.

SEASONAL DEVELOPMENT

Periodic observations were made on the seasonal development of burn
blight on jack pine on various plots in northeastern Wisconsin. The results

are shown in figure 4. In A, trees showing top dying were counted. In B and C the amount of top dying was measured and recorded in percentage of total heights. Most of the trees were from 6 to 14 feet high. In D the dead twigs and branches were removed and their total length was measured. In E the advancing margin down the main stem was determined by gently removing the dead bark scales and by observing the most advanced brown area. This was marked with paint, a different color for successive readings.

Although the total readings gave a clear picture of the extent of the damage, they failed to show the critical time of year for the most rapid development. Consequently the same data were converted to the change per week. Such records appear opposite the others in figure 4.

The total number of trees with top dying (Fig. 4, A) increased from July 2, 1944, to September 13, 1945. The conversion of these data to an increase per week basis (A') shows that the rate of increase was not constant. There were two peaks, one on August 21, 1944, and the other on July 16, 1945, with each peak followed by a sharp decline. The increases were chiefly during the warm moist spring and early summer weather. This is the time when plants are apt to be susceptible. This is likewise ahead of the time when the spittle insects feed on pine. In 1945 the insects were later than usual.

Twenty-nine per cent of the main stems were killed on 145 trees in 1945 in the Phelps area. The increase in downward progress of top dying (Fig. 4, B') was at its peak, 23 per cent per tree per week, on July 16, and declined thereafter. Again the decline in rate of dying was during the period of greatest spittle insect activity. The increase in rate of dying was from April to July. The results of trials on 10-tree plots located near Goodman, Mountain, Phelps, and Stiles, Wis., (Fig. 4, C) agreed closely with those on the 145 trees in the Phelps area (Fig. 4, B). This indicates that the situation at Phelps was not due to local conditions.

Figure 4, D shows a progressive increase in incidence of dead twigs and branches based on foliage symptoms. However, as seen before, the weekly rates of increase (Fig. 4, D') hit peaks in midsummer and declined rapidly in 1945 during the period of spittle insect activity.

The average advance of the necrotic margin showed an increase from October, 1944 to October, 1945 (Fig. 4, E). The rate of weekly increase (Fig. 4, E') showed declines in late summer, which were in accord with the observations on disease manifestations previously described.

A summary of the seasonal development data, as presented in figure 4, indicates that the most rapid disease development occurred during the spring and early summer. The greatest fungus activity was during the same period. This is most easily explained by favorable environmental conditions for the growth of the fungus introduced by a heavy fungus inoculation from the insect vector the previous year.
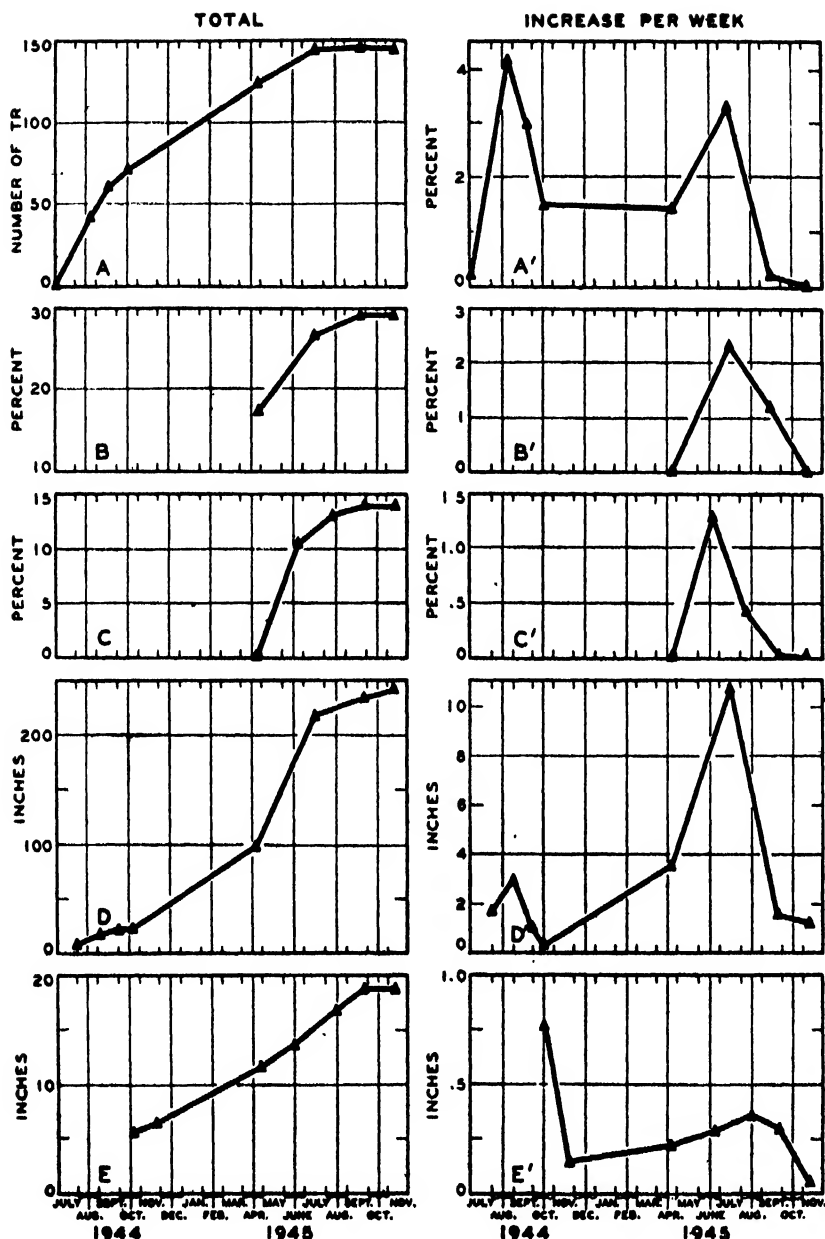
Fig. 4. Summary of disease development on jack pine. A. Number of trees dying near Phelps (6 plots of 1/40 acre each). B. Amount of top dying in percentage of total height (average of 145 trees) as in A. C. Same as B except on 10 trees each near Goodman, Mountain, Phelps, and Stiles. D. Total length of dead twigs and branches near Phelps (average of 18 trees). E. Total necrotic margin advance on main stem, near Goodman, Mountain, and Phelps (average of 214 trees). A', B', C', D', and E' same as A, B, C, D, and E, respectively, except the line in A' is in terms of increase per week, B', C', D', and E' in terms of increase per tree per week.

DISCUSSION

From the evidence presented it appears that the fungus *Chilonectria cucurbitula* associated with burn blight of jack and red pines is pathogenic but is dependent upon some injury, such as the punctures made by the spittle bugs, for entry. The spittle bugs fed on the pines in July, August, and September, inoculating the trees with *C. cucurbitula*. This fungus then killed twigs, branches, and even main stems. It has appeared most active during warm spring weather.

Although *Chilonectria,* if introduced through some other injury, can induce disease independently of the spittle insect, there are indications that the insect alone also can cause serious damage. In any case the two agencies operating together present a serious problem for jack and red pine.

Looking to the future, there appear several possibilities worth considering in relation to control measures. They include: (1) the control of the spittle insect by one or another of the recently developed and powerful insecticides; (2) the selection of sites where trees grow vigorously and, correspondingly, where they may be perhaps less attractive to the spittle insect and less susceptible to the *Chilonectria* than they seem to be on poor sites; and (3) for new plantings it seems desirable to arrange for suitable interplanting with other species or at least for trees in different age classes. Any procedure deserves attention if it tends to reduce the accumulation of large populations either of the spittle insect or of *C. cucurbitula*.

SUMMARY

Jack pine trees affected with burn blight have been dying since 1941 in northeastern Wisconsin and in Michigan. In 1945 there were 64 known disease centers in 9 northeastern Wisconsin counties.

Small twigs at the tops of the trees turn yellow and then brown, and the disease continues downward in the branches and main stems.

Examination revealed that feeding punctures of *Aphrophora saratogensis* Fitch were common on the diseased trees and that necrotic spots caused by *Chilonectria cucurbitula* (Curr.) Sacc. developed around some of the punctures. These brown spots usually enlarged, especially during the warm weather of the following spring and summer, and coalesced, girdling the twigs. Perithecia often developed 4 weeks after inoculation. The fungus moved down the cortex of 10-feet high susceptible trees and killed them in 1 to 3 years.

Chilonectria cucurbitula was isolated, identified, inoculated into trees with positive results, and reisolated. Sixteen different isolates were used in inoculating 412 trees in northern Wisconsin and in the greenhouse or field at Madison.

The adult spittle insect was present from early July until frost. Its feeding punctures appeared commonly on twigs examined in the infected areas. *Chilonectria* was found on every one of 85 adults examined by cul-

tural technique. When adults were placed in a cage, the disease developed on twigs they attacked. Likewise, infection frequently developed about feeding punctures in nature. The evidence suggests that these adults not only acted as vectors but also as agents to weaken the twigs more or less and to provide the *Chilonectria* with a favorable entry.

Vigorously growing twigs, branches, and trees seemed to be relatively resistant and poorly growing trees to be relatively susceptible both to insect inoculation and to puncture inoculation with a spore suspension.

During the growing season feeding by the Saratoga spittle insect occurred in July and August and to a less extent in September. Most of the twig dying appeared with the advent of warm spring and summer weather after the fungus had incubated during the late summer, fall, and early spring.

UNIVERSITY OF WISCONSIN,
    MADISON, WISCONSIN.

LITERATURE CITED

1. BALL, E. D. The spittle insects of the genus *Aphrophora* occurring in the United States. (Homoptera: Cercopidae). Ent. News **45**: 175–179. 1934.
2. ELLIS, J. B., and B. M. EVERHART. The North American Pyrenomycetes. 793 pp. Newfield, N. J. 1892.
3. GRUENHAGEN, R. H. *Hypoxylon pruinatum* and its pathogenesis on poplar. Phytopath. **35**: 72–89. 1945.
4. HENRY, B. W., C. S. MOSES, C. A. RICHARDS, and A. J. RIKER. Oak wilt: its significance, symptoms, and cause. Phytopath. **34**: 636–647. 1944.
5. IVANOFF, S. S. A plant inoculator. Phytopath. **24**: 74–76. 1934.
6. MACALONEY, H. J., and H. C. SECREST. The more common insects attacking young coniferous plantations and natural stands on the national forests of the Lake States and suggestions for preventing or controlling injury. U. S. Dept. Agr., Bur. Ent. and Plant Quar., Div. For. Insect Invest., 15 pp., Mimeo. July, 1944.
7. SACCARDO, P. A. Sylloge Fungorum 2. 815 pp. Friedländer, Berlin. 1883.
8. SECREST, H. C. Damage to red pine and jack pine in the Lake States by the Saratoga spittle bug. Jour. Econ. Ent. **37**: 447–448. 1944.

THE BLACK–POINT DISEASE OF WHEAT

A. F. EL-HELALY

INTRODUCTION

In May, 1940, an outbreak of the black-point disease was first noticed by the writer in the fields of the Ministry of Agriculture at Sakha, Lower Egypt.

Certain preliminary observations were made on this disease. Samples of different varieties of wheat grown in different localities were then carefully examined.

All the material used in this investigation was taken from the experimental fields of the Cereal and the Propagation sections of the Ministry of Agriculture and of the Faculty of Agriculture, Farouk 1 University.

The work to be described in this paper was carried out during 1940–1943 in the laboratories of the Plant Pathology Section of the Ministry of Agriculture, Dokki, and of the Faculty of Agriculture of Farouk 1 University, Damanhour.

HISTORICAL REVIEW

Evans (4) recorded black-point disease of wheat in the Upper Mississippi Valley in 1921. She attributed the disease to a species of *Helminthosporium* resembling *H. sativum* P.K. and B. Curzi (2) found in association with the disease in Italy a species of *Alternaria* not hitherto recorded, and which was named *A. peglionii*. The infesting mycelium does not appear to be limited solely to the teguments of the scutellum, but extends across and under the pericarp and into the groove of the grain, frequently as far as the bearded apex. Rasella (9) mentioned that examination of wheat grains affected with "mouchefure" from Morocco revealed in the discolored regions of the scutellum and the groove of the grain a fairly large, septate, and brown mycelium developing in the teguments but never penetrating deeper. He isolated species of *Alternaria* which he believed to be the same as *A. tenuis* and *A. peglionii*. Pasinetti (8) found that wheat of the awned Argentine variety San Martin was affected with a form of black point. He concluded that the discoloration of the scutellum was not due to an organism. The condition was regarded as purely physiological and resulting from adverse environmental (perhaps climatic) conditions. Ziling (11) stated that a disease similar to that known in Italy as "puntatura" and in Morocco as "moucheture" is known to have occurred in Siberia at least since 1914. Isolations from diseased grains germinated on filter paper yielded *Alternaria tenuis* in 82 to 95 per cent, *Helminthosporium sativum* in 15 to 60 per cent, species of *Fusarium* in about 4 per cent, and a number of unidentified fungi in some 2 per cent of the cases. Bacteria were not present. Laumont and Murat (5) stated that microscopical examination of clean and affected grains

belonging to a number of wheat varieties of different origins showed the almost constant presence in their external integuments of a mycelium which in culture usually produced a species of *Alternaria*, believed to be *A. peglionii*. *Helminthosporium sativum* has never been found on wheat in Algeria. Dastur (3) stated that a condition of wheat grains closely resembling that described as black point in the Argentine San Martin wheat by Pasinetti in 1931 occurred in the Central Provinces of India. The only important point of difference is that while no organisms were found by Pasinetti in the discolored grains, bacteria and species of *Helminthosporium*, *Cladosporium*, *Ophiobolus*, and *Fusarium* were shown to be present in the Indian wheats. In a few cases, however, black-point wheat grains failed to show the presence in them of any organism. Hyphae were observed inside the affected grains in great abundance in the funicle and in the pericarp in the central region of the grain furrow; they grow between the pericarp and the seed coat, where they form a kind of stroma, and also occur in the coleoptile and in crushed cells behind the scutellum. Machacek and Greaney (6) noticed that the disease was prevalent and of economic importance in Manitoba and that apparently *durum* wheats were more susceptible to it than *vulgare* varieties. It was further found that the fungi chiefly associated in Manitoba with this condition are *Alternaria tenuis*, *A. peglionii*, *Helminthosporium sativum*, and *H. teres*. They suggest the term "kernel smudge" for the condition. Connors (1) recorded that the disease, due chiefly to species of *Alternaria*, was more prevalent than usual in the Prairie Provinces of Canada. The condition caused the de-grading of 7.6 per cent of the ears in Manitoba, 2.4 per cent in Saskatchewan, and of 2 ears in 5000 in Alberta.

SYMPTOMS OF THE DISEASE

The black-point or kernel-smudge disease of wheat is characterized by a brown diffuse discoloration of varying intensity, limited to the embryo region in the grain. It, often, has a narrow brown line extending along the groove, in some cases to the brush end of the kernel.

CAUSE OF THE DISEASE

Numerous isolations from superficially disinfected diseased grains in Egypt have given a species of *Alternaria*.

On examining sections made at the discolored parts of the grain, mycelium was seen in a few cases. It was located in the pericarp and penetrated to the integument. No invading hyphae have been seen in the aleurone layer, the starch cells of the endosperm, or the embryo.

The same fungus was also isolated from a few superficially sterilized kernels that were apparently healthy.

OCCURRENCE AND DISTRIBUTION OF THE DISEASE.

The black-point disease has probably been present in Egypt for a long time, though there is no mention of the disease in the Egyptian literature.

Examination of samples of different varieties of wheat grain grown in different localities all over Egypt during 1940–1943 showed that the disease, in some of the varieties tested, is prevalent in the northern part of Lower Egypt and that the intensity of the disease decreases gradually southwards becoming rare in Upper Egypt.

In Giza 114, one of the very susceptible varieties, we find that the percentage of the disease in the crop of May, 1940, amounted to about 40 in the northern part of Lower Egypt, 30 in the southern part, 12 in Middle Egypt, and 1.5 in Upper Egypt.

The intensity of the disease varies from year to year and with the different varieties of wheat.

When the disease is severe and prevalent it becomes of some economic importance because it lowers greatly the grade of the grain. The disease has no harmful effect on the germination of the affected seeds, the emergence of the seedlings, or the yield of the subsequent crop.

FACTORS INFLUENCING DISTRIBUTION OF THE DISEASE

The spread and distribution of the disease in Egypt depend on a number of factors which act either together or separately. Some of these factors are external while the others are internal.

External Factors

Presence of the Spores of the Casual Organism in the Air. Infection, in this disease, results from air-borne spores which come in contact with the exposed part of the grain when conditions are favorable. This was ascertained by keeping certain immature ears of some susceptible plants under controlled conditions to prevent infection from the air as much as possible. When these were ripe the grains were apparently clean while the rest of the ears which were left to ripen naturally while exposed to the air contained some discolored grains.

Machacek and Greaney (6) working on this disease stated that the cereal grains, under Manitoba conditions, are infected by air-borne spores of the fungi. It was demonstrated by means of spore-traps that the largest numbers of such spores usually occur in the air about the time the grains are maturing.

Atmospheric Humidity and Rainfall. Atmospheric humidity and rainfall seem to favor the occurrence of the disease. There is comparatively more rain in the northern part of Lower Egypt than in the southern part, while there is very little rain in Middle Egypt, and practically no rain in Upper Egypt. The intensity of the disease in Egypt follows that of the rainfall. Table 1 contains the percentage of the disease in different localities as well as the amount of rainfall and the relative humidity in each of these localities.

Soil Humidity. There is no doubt that the amount of water given to the soil during the growing period of the crop influences the incidence of the

disease. It was observed by Pantanelli (7) that infection was very severe in cases where much water was absorbed by the plant, in proportion to the weight of its absorptive system, during the period immediately preceding inoculation. He also states that an increased concentration of the nutrient solution diminishes susceptibility in so far as it reduces the absorptive activity of the roots.

In Assiut Province in Egypt two systems of irrigation are applied. One area of this province is under the perennial system of irrigation: the crop is given more than one watering during its growing season. The other area is

TABLE 1.—*The percentage of black point in two varieties of wheat and relative humidity and monthly rainfall at different localities in Egypt in 1941*

Locality	Percentage black-point infection		Mean daily relative humidity (per cent)			Monthly rainfall (mm.)		
	Giza 114	Baladi 116	Mar.	Apr.	May	Mar.	Apr.	May
Sakha	62.5	20.0	84	72	62	22.2	Tr.	0.0
Damanhour	50.0	18.0	77	69	57	23.1	0.1	0.0
Mansura	50.0	13.0	80	69	57	27.0	Tr.	0.0
Gemmeza	31.5	12.0	88	76	71	19.2	Tr.	0.0
Giza	16.5	8.0	68	59	48	9.5	Tr.	Tr.
Minya	11.0	6.0	52	43	36	Tr.	Tr.	0.0
Assiut	1.5	1.5	51	39	30	0.0	0.0	0.0
Quena	1.5	1.0	42	32	24	0.0	Tr.	Tr.
Aswan	2.0	1.0	21	16	11	0.0	3.0	Tr.

basin lands and is under the flooding system of irrigation: the crop receives only one watering at the time of sowing. In the first area the incidence of the disease is rather higher and more noticeable than in the latter area where the disease is very light and even rare.

The data in table 2 were taken from two experiments, one in Mallawi which falls within the first area and the other in Rifa, a village, close to Assiut, which lies within the second area.

TABLE 2.—*The percentage of black point in four varieties of wheat grown at Mallawi where there were several irrigations during the season and at Rifa where a single flooding irrigated the crop*

Variety	Percentage of infection at	
	Mallawi	Rifa
Giza 114	12.5	1.5
Baladi 116	7.5	1.5
Giza 121 (Mabrouk)	4.5	1.5
Hindi 62	3.5	1.5

Soil humidity favors the disease attack. It also appears from the results in table 2 that the intensity of the disease varies with the different varieties of wheat.

Internal Factors

Size of Wheat Grain. It has been observed during this investigation that the size of wheat grain plays an important part in the incidence of the disease. Larger grains show usually heavier attack. Ziling (11) mentioned that wheat grains infected with *Alternaria tenuis* were readily distinguishable from those infected with *Helminthosporium sativum* in that the former were usually larger and heavier, while the latter were smaller and lighter than usual. Waldron (10) recorded that the weight of the black-point kernels on any one plant was generally greater than that of the apparently non-infected kernels. These results are considered to indicate that a portion of the weight differences is caused by a stimulation of the development of the endosperm, following the penetration of the fungus into the developing ovule, although they may also be attributed in part to a difference in infection of grains differing normally in size because of their position in the ear. Machacek and Greaney (6) stated that the largest grains in the ear are frequently infected while the small, shrunken ones are usually free from the disease.

VARIETAL RESISTANCE UNDER NATURAL INFECTION

Some varieties appear to be more susceptible to black-point disease than others (Table 2). A more extensive test on a number of varieties was carried out.

Grain samples of different varieties of wheat were taken from the crops resulting from many variety experiments made all over Egypt. Examination of these samples showed that some of the varieties tested were more affected than the others. Data on grain samples from two such experiments, at Sakha and at Gemmeza in Lower Egypt where the disease is prevalent, appear in table 3.

TABLE 3.—*The percentage of black point in grain samples of several varieties of wheat grown at two locations in Lower Egypt where the disease is prevalent*

Variety	Percentage of infected grains in samples from	
	Sakha[a]	Gemmeza[a]
Giza 114	62.5	31.5
Giza 115	60	20
Giza 116	44	20
Giza 74	48	12
Baladi 116	20	12
Giza 121 (Mabrouk)	12	8
Giza 100	13	3
Hindi 62	12	2
Giza 102	10	3
Giza 7	10	3
Hindi D	5	2

[a] Sakha is in the northern part of Lower Egypt, Gemmeza is in the southern part.

Hence, among the 11 varieties examined the least susceptible to black-point disease is Hindi D followed by Giza 7, Giza 102, and Giza 121 (Ma-

brouk). The varieties Giza 114, Giza 115, Giza 116, and Giza 74 are the most susceptible. Baladi 116 is partially susceptible.

MECHANISM OF RESISTANCE

The statement of Machacek and Greaney (6) may explain the mechanism of resistance to black-point disease in wheat. They stated that the largest grains in the ear are frequently infected while the small, shrunken ones are usually free from infection. This apparently is due to the fact that the large kernels force open their covering glumes, thus affording access to the spores, whereas the glumes of the small kernels remain closed and exclude the spores.

The volume of a known number of grains of certain resistant and susceptible wheat varieties was measured, and the percentage of the disease was estimated in each variety. The data obtained are in table 4.

TABLE 4.—*The volume of 1000 kernels and the percentage of black point in seven varieties of wheat*

Variety	Av. volume (cc.) of 1000 kernels	Percentage of infection
Giza 114	39	60
Giza 116	39	45
Giza 74	37	45
Baladi 116	30	22
Giza 121 (Mabrouk)	27	13
Hindi 62	28	13
Hindi D	25	5

The results illustrate that the most susceptible varieties, Giza 114, Giza 116, and Giza 74, have large kernels. Kernel volume is least in the most resistant variety, Hindi D; while the moderately susceptible variety Baladi 116 has an intermediate kernel volume.

Diseased and apparently healthy grains found in samples of certain varieties of wheat were separated from each other. The volume of each lot in each variety was determined. The results are recorded in table 5. The diseased grains were invariably larger than the apparently healthy ones.

TABLE 5.—*The volume of 1000 healthy kernels and of 1000 black-point kernels in grain samples of wheat varieties grown at Giza in 1941–42 and at Damanhour in 1942–43*

Variety and source of sample	Black point (per cent)	Av. volume (cc.) of 1000 kernels	
		Healthy	Infected
Baladi 116			
Giza, 1941–42	7.5	30.0	40.0
Damanhour, 1942–43	21.0	29.5	38.5
Hindi 62			
Giza, 1941–42	4.5	25.0	35.0
Giza 121			
Giza, 1941–42	1.5	25.0	35.5
Damanhour, 1942–43	1.0	27.5	40.0

It is, therefore, clear, that the size of the grain is directly associated with the incidence of the disease. Resistance of some varieties of wheat to black-point disease is a morphological condition depending on the grains and their covering glumes. Resistant varieties produce small grains which have no effect on the glumes. The glumes, therefore, remain closed and do not permit the passage of the spores to the grain. Thus, infection is avoided even when all external conditions favor it.

It has been noticed, in some cases, that percentage of infection may vary in the same variety even when grown in the same locality. Investigation has shown that rust disease is the limiting factor in this case. It affects the grains which become small and shrunken and thus avoid infection by black-point disease so that the variety gains resistance.

SUMMARY

Black-point disease of wheat in Egypt was found to be due to species of *Alternaria*.

The disease is widely distributed in Lower Egypt. It decreases gradually southwards becoming rare in Upper Egypt.

The disease has no harmful effect on the germination of the affected grain. It only lowers the grade.

The spread and distribution of the disease in Egypt depend on a number of factors. The most important of these factors are presence of the spores of the casual organism in the air, atmospheric humidity and rainfall, soil humidity, and size of the grain.

Of the varieties of wheat tested with regard to differential susceptibility, Hindi D proved to be the least susceptible followed by Giza 7, Giza 102, and Giza 121 (Mabrouk). Giza 114, Giza 115, Giza 116, and Giza 74 were the most susceptible. Baladi 116 is partially susceptible.

The size of the grain affects directly the incidence of the disease. The large kernels force open their covering glumes, thus affording access to the spores, whereas the glumes of small kernels remain closed and, thus, avoid infection even when all conditions favor it.

The resistance of some varieties of wheat is, mainly, due to the fact that they produce small grains.

FACULTY OF AGRICULTURE,
FAROUK 1 UNIVERSITY,
ALEXANDRIA, EGYPT.

LITERATURE CITED

1. CONNERS, I. L. Twentieth Annual Report of the Canadian Plant Disease Survey, 1940. XVI + 104 pp., 1941.
2. CURZI, M. La "puntatura" della cariossidi di frumento e una nuova specie di Alternaria. Riv. Pat. Veg. 16: 125–136. 1926.
3. DASTUR, J. F. Microscopic characters of the black point disease of wheat in the Central Provinces. Proc. World's Grain Exhib. Conf., 1933. Regina, 2: 253–255. 1935.
4. EVANS, NEVADA S. "Black point" of wheat. Phytopath 11: 515. 1921.
5. LAUMONT, P., and M. MURAT. Observations sur la moucheture et la mauvaise germination de quelques blés en 1933. Bull. Soc. Hist. Nat. Afrique du Nord, 25: 253–265. 1934.
6. MACHACEK, J. E., and F. J. GREANEY. The "black point" or "kernel smudge" disease of cereals. Canad. Jour. Res., (C) 16: 84–113. 1938.
7. PANTANELLI, E. Sui rapporti fra nutrizione e recettivita per la ruggine. Riv. Pat. Veg., 11: 36–64. 1921.
8. PASINETTI, L. Ricerche anatomo-fisiologiche sulla "puntanera" del frumento argentino "San Martin." Riv. Pat. Veg., 21: 145–156. 1931.
9. RASELLA, E. Quelques observations sur la moucheture des céréales. Rev. Path. Vég. et Ent. Agric., 17: 338–344. 1930.
10. WALDRON, L. R. Increase of kernel weight in common wheat due to black-point disease. Jour. Agr. Res., [U.S.] 48: 1017–1024. 1934.
11. ZILING, M. K. ["Black germ" of wheat.] Diseases of Cereal Crops. Siberian Scient. Res. Inst. Omsk. pp. 15–39. 1932..

A STRAIN OF THE ALFALFA MOSAIC VIRUS ON PEPPER IN ONTARIO[1]

G. H. BERKELEY[2]

In Brant and Lincoln counties, in 1943 and again in 1944, an unusual mosaic disease of pepper (California Wonder variety) was observed. In addition to mottling, chlorotic rings, spots, and patterns appeared on both leaves and fruit. These symptoms suggested that a virus was involved other than those found commonly on pepper in Ontario, namely, tobacco mosaic and cucumber mosaic. Preliminary inoculations indicated that a strain of *Marmor medicaginis* H. var. *typicum* was responsible for the unusual symptoms. So far as the author is aware, this is the first occurrence of this disease appearing naturally on sweet peppers.

EARLIER INVESTIGATIONS

Porter (5, 6) and Dykstra (2) have reported pepper as a host of the potato-calico virus, which Black and Price (1) consider to be *Marmor medicaginis* var. *solani*. Though Zaumeyer (8) and Zaumeyer and Wade (9, 10), have made comprehensive studies of the alfalfa-mosaic viruses, they did not include pepper in their range of hosts. In 1942, *M. medicaginis* was reported by Kovacevski (3) as occurring on Chili peppers in Bulgaria and by Snyder and Rich (7) on celery in California.

MATERIAL AND METHODS

Several isolates of pepper virus were obtained from naturally affected peppers in both Brant and Lincoln counties. As those from all sources produced similar symptoms on hosts within a restricted range, one was chosen for use throughout this investigation. For comparative purposes *Marmor medicaginis* H. var. *typicum* and *M. medicaginis* var. *solani*, the potato-calico strain, were kindly supplied by Dr. F. O. Holmes.

The differential host range included seedlings of *Nicotiana tabacum* L., var. Harrow Velvet; *N. glutinosa* L.; *N. rustica* L.; *Vicia faba* L., broad bean, var. Windsor Long Pod; *Phaseolus vulgaris* L., bean, var. Stringless Evergreen; *Lathyrus odoratus* L., sweet pea, var. Pinkie; *Pisum sativum* L., pea, var. Laxton's Progress or Dwarf Telephone; *Trifolium pratense* L., red clover; *Capsicum frutescens* L., pepper, var. California Wonder; *Soya max* (L.) Piper, soybean, var. Blackeye; *Lycopersicon esculentum* Mill., tomato, var. Grand Rapids; *Datura stramonium* L.; *Antirrhinum majus* L., snapdragon, var. Empress; *Solanum Melongena* L., eggplant, var. Black Beauty; *Cucumis sativus* L., cucumber, var. Windermoor Wonder; *Petunia*
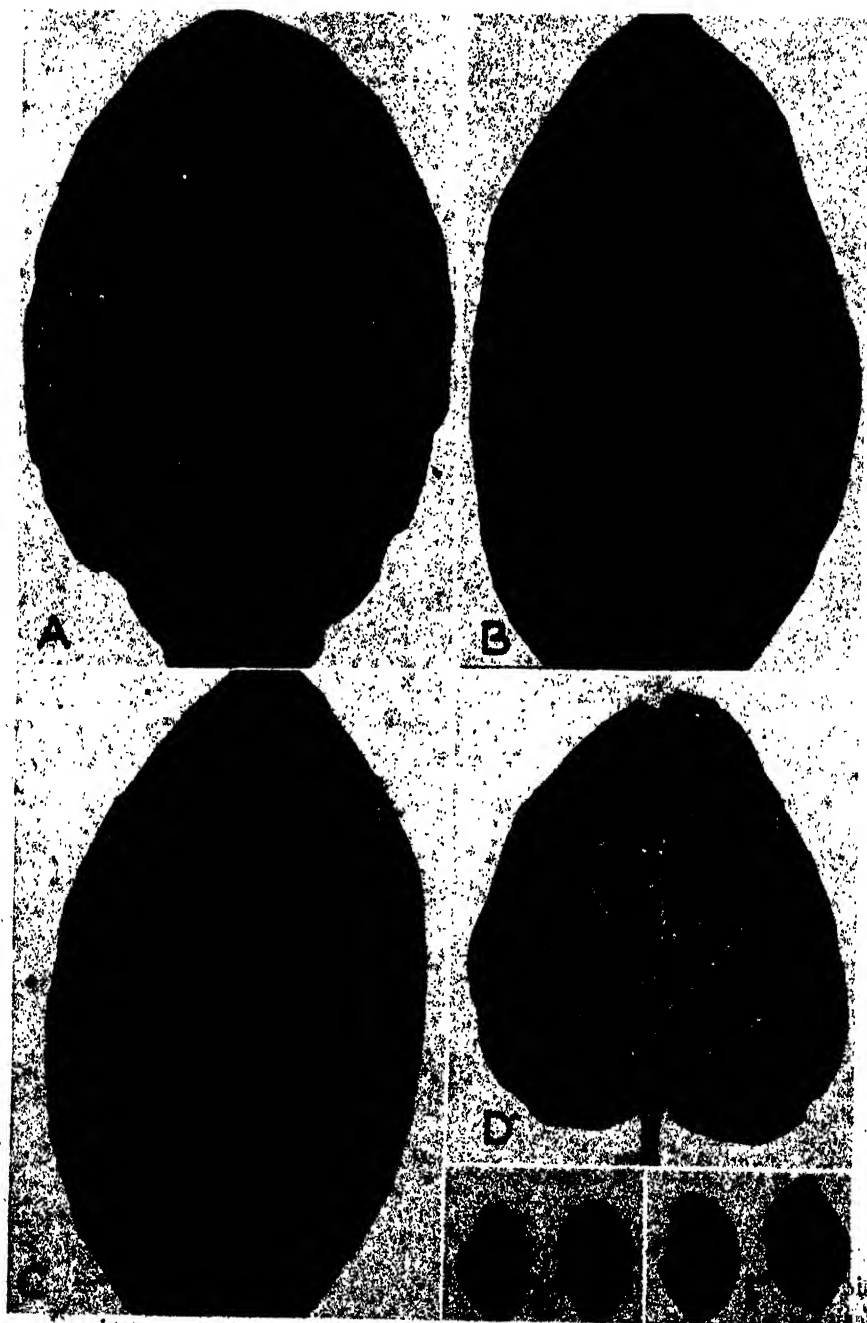
Fig. 1. Symptoms of pepper virus on various hosts. A: necrotic spots and rings on *Nicotiana tabacum*; B: systemic flecking on *N. tabacum*; C: vein-banding on *N. tabacum*; D: chlorotic and necrotic spots and rings on *N. glutinosa*; E: necrotic local lesions on sweet pea; F: systemic necrosis on sweet pea.

hybrida Viln., petunia; *Zinnia elegans* Jacq., zinnia; and *Apium graveolens* L., celery, var. Golden Plume.

Rapidly growing seedlings of a 4- to 6-leaf stage were generally used for inoculation which was effected by the cotton swab-carborundum technique, using unfiltered juice obtained by crushing fresh leaf material in a mortar and pestle.

Inoculations extended over a 3-year period, so that a wide range of environmental conditions was encountered.

SYMPTOMS PRODUCED BY THE PEPPER VIRUS

Before discussing the symptoms in detail for each host, it should be pointed out that there was considerable variation in this regard. For instance, only primary yellow lesions developed on the inoculated leaves in 2 series though in 14 series necrotic spots and rings were also formed. In some series both necrotic spots and rings developed, whereas in others only chlorotic or necrotic rings resulted. The most important single factor in this connection appeared to be the age of the rubbed leaves. For instance, in several series where the lowest leaf and two leaves above in order were inoculated on the same plant (*Nicotiana tabacum*), the symptoms on the bottom leaf were of the solid necrotic lesion type while, on the two upper leaves, primary yellow lesions with grayish-white necrotic rings developing around the primary yellow lesion were general, with solid necrotic lesions usually absent. The same effect was obtained on *N. rustica* when the lower leaf showed large, black, solid necrotic lesions while on the upper leaves smaller white papery necrotic lesions were formed.

Nicotiana tabacum. Sixteen series of inoculations involving 60 plants were carried out on this host. The general symptoms were primary yellow lesions with or without necrotic centers and grayish white necrotic rings followed by systemic vein-clearing, vein-banding, chlorotic mottling, and white necrotic flecks, rings and/or patterns (Fig. 1, A, B, and C). On plants held for several weeks a chlorotic mottling with some necrotic flecking was the predominant symptom on the later formed leaves.

When old slow-growing plants were inoculated, the symptoms were often very mild, with yellow lesions as the primary symptom and little or no necrotic flecking.

Nicotiana glutinosa. Fourteen series of inoculations, involving 52 plants, were effected on *N. glutinosa.* The resulting symptoms consisted of systemic vein-clearing, vein-banding, and a green-spot type of mottle with necrotic flecks and lines. On the rubbed leaves the symptoms varied considerably. Sometimes symptoms were lacking while at other times yellow ons, which generally became necrotic, developed before, at about the same me, or after the first systemic symptoms (Fig. 1, D). On plants held for several weeks, chlorotic mottling with mild necrosis became the predominant symptom on later formed leaves.

Nicotiana rustica. On this host fifteen series of inoculations, involving 55 plants, were effected. Symptoms consisted of primary yellow lesions, grayish-white etched rings, and/or concentric rings, followed by systemic
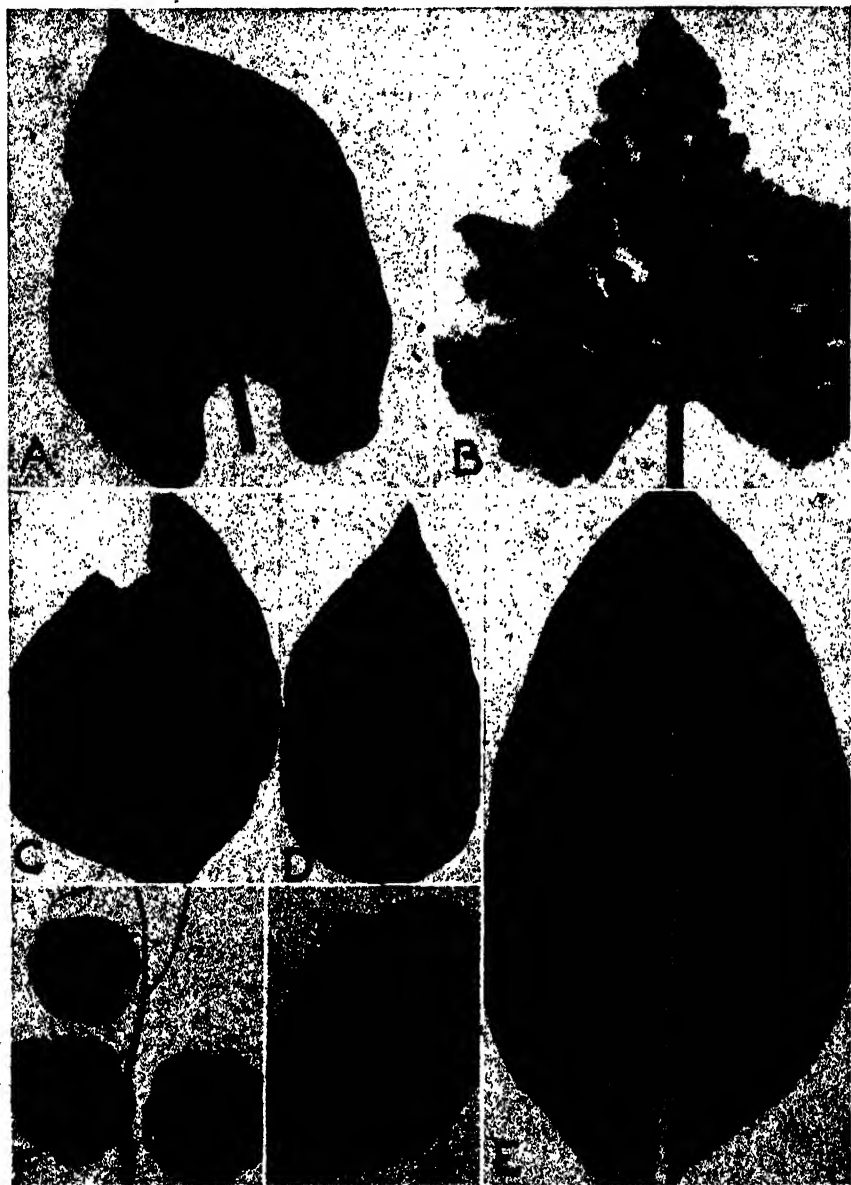
FIG. 2. Symptoms of pepper virus. A: necrotic spotting on bean; B: typical chlorotic vein-banding type of mottle on cucumber; C: chlorotic mottle and patterns on pepper; F: mottle on pea; G: necrotic lesions on broad bean. D and E: symptoms of potato-calico virus on pepper and *Nicotiana rustica.* Note wide vein-banding on *N. rustica* and lack of chlorotic-patterns on pepper. The three spots in D were caused by film defects and are not symptoms of virus infection.

vein-banding, chlorotic mottle, chlorotic rings, necrotic flecks, and necrotic line patterns associated with the larger veins.

Snap bean var. Stringless Evergreen. Four series of inoculations, involving 20 plants, were carried out on this host. Only primary symptoms were obtained, consisting of small dark necrotic lesions (Fig. 2, A).

Windsor Broad Bean. Four series of inoculations, involving 20 plants, were effected. Symptoms consisted of black primary necrotic lesions followed by systemic necrosis and necrotic spotting. (Fig. 2, G) sometimes resulting in the death of the plants. A mild chlorotic mottle was present in some cases where the necrosis was not severe.

Soybean var. Blackeye. Three series of inoculations, involving 10 plants, were carried out. All inoculated plants developed a mild systemic mottle, with no primary symptoms.

Datura stramonium. On this host, five series of inoculations, involving 25 plants, were effected. Symptoms consisted of primary yellow lesions, followed by systemic mild mottle with fine grayish-white etched rings and sometimes mild vein-banding.

Field pea var. Laxton's Progress. Twelve series of inoculations, involving 58 plants, gave somewhat inconsistent results in that the percentage of successful inoculations was at times very low. At other times infection was one hundred per cent. The first symptom was a drying out of the rubbed leaves. This was followed by a general wilting and stunting of the plant, with mild mottling of tip leaves (Fig. 2, F). In some cases, only tip leaves wilted, while in others, the plants were killed. The foliage became light green to yellow and the general appearance of affected plants suggested root injury rather than symptoms resulting from virus infection. Accordingly, transfers were made from wilted tip leaves of pea to *Nicotiana tabacum* and *N. glutinosa* resulting in symptoms typical for the pepper virus, thus indicating that these unusual symptoms on pea were due to infection by the virus.

Sweet pea var. Pinkie. Eleven series of inoculations, involving 43 plants, were carried out on sweet pea. Primary symptoms consisted of solid necrotic "tear drop" lesions on the rubbed leaves, which later were cast (Fig. 1, E). Systemic symptoms, which were not general on a plant, comprised curling and dwarfing of tip leaves, while only a few leaves developed chlorotic spotting or white necrotic streaks or stippling, generally towards the base of affected leaves (Fig. 1, F). All plants were stunted and, in some cases, were killed. Often one lateral shoot was killed while the remainder of the plant continued to grow, though growth was severely stunted.

Red clover. On this host seven series of inoculations, comprising 32 plants, gave positive results in only 3 series. Accordingly, it would appear that red clover is not readily infected by the pepper virus. Symptoms, when obtained, consisted of general mottling with slight distortion of leaves. In one series large brown necrotic lesions developed on the rubbed leaves.

Cucumber var. Windermoor Wonder. Fourteen series of inoculations, comprising 52 plants, gave inconsistent results in that in six series negative results were secured. In eight series, however, symptoms consisting of general mottling of a chlorotic vein-banding and asteroid-like type were consistently obtained (Fig. 2, B). Affected plants became stunted and the fruit showed chlorotic spots and rings with little or no distortion.

Pepper var. California Wonder. Only 4 series of inoculations, involving 24 plants, were tested on this host. All plants showed similar symptoms, consisting of general mottle with chlorotic rings, spots, and often chlorotic line patterns (Fig. 2, C).

Snapdragon var. Empress. In this case also only 4 series of inoculations, comprising 22 plants, were tested. Symptoms consisted of reddish rings or patterns on rubbed leaves, and sometimes were more readily apparent on the lower than the upper surface of the leaf. Transfers from such leaves to *Nicotiana tabacum* and *N. glutinosa* produced symptoms on these hosts typical for the pepper virus. No definite systemic symptoms were apparent.

Tomato var. Grand Rapids. Inoculations in six series, and involving 28 plants, gave negative results. Inoculations from tomato back to *Nicotiana tabacum* or *N. glutinosa* gave no symptoms, indicating that the tomato plants were not acting as "carriers" of the virus. It would appear therefore that tomato is not a host of the pepper virus.

Petunia. The symptoms on petunia consisted of dark green rings on rubbed leaves and systemic vein-clearing or general vein-banding on the upper leaves.

Zinnia. In eight series of inoculations, comprising 34 plants, rather inconsistent symptoms were obtained. The first sign of infection was the turning downward of certain leaves, or a temporary twisting of the tip of the plant. Primary symptoms consisting of yellow lesions were sometimes present, sometimes absent. Systemic mottling was largely confined to the newer leaves. In many cases the mottle was indistinct, while in others it was definite and of the spot type.

Celery var. Golden Plume. On celery five series of inoculations, involving 20 plants, were effected. A chlorotic mottle, especially on the older leaves, general stunting, and mild distortion of leaflets were the only symptoms. The chlorotic areas were usually more numerous towards the margins of the leaf. In time much of the chlorotic tissue faded to almost white.

Eggplant, var. Black Beauty. Only a few inoculations were made on eggplant but all produced a general systemic mottle.

COMPARISON OF THE SYMPTOMS CAUSED BY THE PEPPER VIRUS WITH THOSE CAUSED BY THE ALFALFA-MOSAIC VIRUS AND THE POTATO-CALICO STRAIN

The same number of series of inoculations, as effected for the pepper virus, were also carried out on the same hosts with *Marmor medicaginis* var.

typicum and *M. medicaginis* var. *solani*. This was necessary since many of the descriptions of symptoms given in the literature for alfalfa mosaic and potato calico are meagre and insufficient for comparative purposes.

In general, it was found that the alfalfa-mosaic and potato-calico viruses produced symptoms of the same general type as those associated with the pepper virus. For instance, on tobacco, broad bean, bean, sweet pea, pea, soybean, *Datura stramonium*, snapdragon, eggplant, petunia, celery, and zinnia, all three viruses produced similar symptoms, differing at times in degree only. However, the symptoms on certain hosts differed to a sufficient degree to suggest that the potato-calico virus and the pepper virus should be considered as distinct strains of the alfalfa-mosaic virus.

The pepper virus produced more necrosis on *Nicotiana tabacum*, *N. glutinosa*, and *N. rustica* than did either the alfalfa-mosaic virus or the potato-calico virus. In this respect, the alfalfa-mosaic virus was intermediate between the pepper virus and the potato-calico virus, which produced the least necrosis. The pepper virus did not produce symptoms on tomato, whereas this host was readily infected by both the alfalfa-mosaic and the potato-calico viruses. The potato-calico virus produced more vein-clearing and fine vein-banding than either of the other viruses. On red clover both the alfalfa-mosaic and pepper viruses produced mottling with distortion of leaves, whereas distortion was, for the most part, lacking with the potato-calico virus.

The potato-calico virus caused a pronounced blotchy mottle on pepper (Fig. 2, D), whereas, with the alfalfa-mosaic and pepper viruses, the mottle was finer and chlorotic rings were more common. Whereas the potato-calico virus produced a coarse blotchy mottle with prominent wide vein-banding on *Nicotiana glutinosa* and *N. rustica* (Fig. 2, E), the alfalfa-mosaic virus and the pepper virus produced only a chlorotic mottle with rings, lines, etc.

No infection was obtained on cucumber with the potato-calico virus whereas this host was readily infected by the alfalfa-mosaic and pepper viruses.

As to necrosis on broad bean it was least with the potato-calico virus and greatest with the pepper virus.

CROSS PROTECTION TESTS

To verify further the close relationship of these viruses, *Nicotiana tabacum* plants systemically infected with Nicotiana virus 1, Cucumis virus 1, alfalfa-mosaic virus, and the potato-calico virus were re-inoculated with the pepper virus by rubbing one half of two or three leaves on each plant. No symptoms developed on the rubbed portions of leaves on the plants previously infected with the alfalfa-mosaic virus or the potato-calico virus, but typical local lesions and chlorosis developed on the rubbed leaves of the plants systemically infected with Nicotiana virus 1 and Cucumis virus 1. These results indicate that the pepper virus is closely related to the alfalfa-mosaic virus.

The thermal death point of the pepper virus was between 63° C. and 64° C. Some tests at 62° C. were positive and some negative. All tests at 61° C. were positive while at 63° C. and 64° C. all were negative.

According to Pierce (4) and Zaumeyer and Wade (10), the thermal death point of alfalfa virus 1 is between 62° C. and 65° C., while Zaumeyer (8) found it to be between 65° C. and 70° C.

Therefore the thermal death points for the alfalfa-mosaic virus and the pepper virus are in close agreement.

DISCUSSION

The symptoms produced by the pepper virus on 17 of the 18 hosts used in this investigation were in close agreement with symptoms associated with the alfalfa-mosaic virus on the same hosts. Moreover, the cross protection and thermal death point tests also indicated that the pepper virus was closely related to the alfalfa-mosaic virus. However, the fact that the pepper virus did not infect tomato and that it consistently produced more necrosis than the alfalfa-mosaic virus, would suggest that the pepper virus should be considered to be a strain of the alfalfa-mosaic virus.

The cross protection tests also indicated that the potato-calico virus was closely related to the alfalfa-mosaic virus. However, the negative results on cucumber, and the slightly different symptoms produced by the potato-calico virus on *Nicotiana glutinosa*, *N. rustica*, and pepper in comparison with those associated with the alfalfa-mosaic virus and the pepper virus on these same hosts, would suggest that the potato-calico virus also should be considered a distinct strain of alfalfa-mosaic virus. These results are, therefore, in agreement with those of Black and Price (1), who named the potato-calico virus *Marmor medicaginis* var. *solani*.

It is suggested that the pepper virus be called *Marmor medicaginis* var. *capsici*.

SUMMARY

An unusual type of mosaic was found on sweet pepper var. California Wonder in Ontario in 1943 and again in 1944. Symptoms included chlorotic rings, spots, and patterns on both leaves and fruit.

A large number of inoculations, extending over a 3-year period, using *Marmor medicaginis* H. var. *typicum*, *M. medicaginis* H. var. *solani* and the pepper virus indicated that the pepper virus was a strain of *Marmor medicaginis*. Comparative studies were made on *Nicotiana tabacum* L.; *N. rustica* L.; *N. glutinosa* L.; *Vicia faba* L., broad bean; *Lathyrus odoratus* L., sweet pea; *Pisum sativum* L., pea; *Phaseolus vulgaris* L., bean; *Trifolium pratense* L., red clover; *Capsicum frutescens* L., pepper; *Soja max* (L.) Piper, soybean; *Solanum Melongena* L., eggplant; *Zinnia elegans* Jacq., zinnia; *Cucumis sativus* L., cucumber; *Petunia hybrida* Viln., petunia; *Lycopersicon esculentum* Mill., tomato; *Antirrhinum majus* L., snapdragon; and *Apium graveolens* L., celery.

With the following exceptions, all 3 viruses produced similar symptoms on the above hosts. The pepper virus did not infect tomato, while both the alfalfa-mosaic virus and the potato-calico strain did. Whereas, the pepper virus and the alfalfa-mosaic virus gave positive results on cucumber, negative results were obtained with the potato-calico virus. The potato-calico strain produced prominent calico symptoms with wide vein-banding on *Nicotiana rustica* and *N. glutinosa*. On these same hosts, alfalfa-mosaic and pepper viruses produced much less prominent symptoms, with the wide vein-banding lacking. The pepper virus produced more severe necrosis on *N. glutinosa*, *N. rustica*, and *N. tabacum* than did the alfalfa-mosaic virus or the potato-calico virus.

Cross protection and thermal death point tests also indicated that the pepper virus was a strain of *Marmor medicaginis* H.

It is suggested that the pepper virus be called *Marmor medicaginis* var. *capsici*.

DOMINION LABORATORY OF PLANT PATHOLOGY,
 ST. CATHARINES, ONTARIO.

LITERATURE CITED

1. BLACK, L. M., and W. C. PRICE. The relationship between viruses of potato calico and alfalfa mosaic. Phytopath. 30: 444–447. 1940.
2. DYKSTRA, T. P. A study of viruses causing yellow mosaics in European and American varieties of the potato *Solanum tuberosum*. Phytopath. 29: 917–933. 1939.
3. KOVACEVSKI, I. C. Die Buntblättrigkeit der Paprikapflanze (*Capsicum annuum*) (*Medicago* virus 2 K. Smith var. *typicum* Black & Price). Vorlaufige Mitteilung. Ztschr. Pflkrankh. 52: 533–540. 1942. Abstr. in Rev. Appl. Mycol. 22: 275. 1943.
4. PIERCE, W. H. The identification of certain viruses affecting Leguminous. plants. Jour. Agr. Res. [U.S.] 51: 1017–1039. 1935.
5. PORTER, D. R. The infectious nature of potato calico. Hilgardia 6: 277–294. 1931.
6. ————. Insect transmission, host range and field spread of potato calico. Hilgardia 9: 383–394. 1935.
7. SNYDER, W. C., and S. RICH. Mosaic of celery caused by the virus of alfalfa mosaic. Phytopath. 32: 537–539. 1942.
8. ZAUMEYER, W. J. A streak disease of peas and its relation to several strains of alfalfa mosaic virus. Jour. Agr. Res. [U.S.] 56: 747–772. 1938.
9. ————, and B. L. WADE. The relationship of certain legume mosaics to bean. Jour. Agr. Res. [U.S.] 51: 715–749. 1935.
10. ————, and ————. Pea mosaic and its relationship to other legume mosaic viruses. Jour. Agr. Res. [U.S.] 53: 161–185. 1936.

METHODS FOR DETERMINING RESISTANCE OF OATS TO HELMINTHOSPORIUM VICTORIAE[1]

S. C. LITZENBERGER AND H. C. MURPHY[2]

INTRODUCTION

Helminthosporium blight of oats caused by *Helminthosporium victoriae* Meehan and Murphy[3,4,5,6] was first observed in the field in 1945. In 1946, it had become a major disease of oats in Iowa, Illinois, Indiana, New York, and certain other states. A large portion of the oat acreage in the United States was sown to crown rust resistant varieties that had been derived from Victoria (C.I. 2401[7]), a variety introduced from South America in 1927. Susceptibility to *H. victoriae* has been inherited with the "Victoria type" of resistance to *Puccinia coronata* Corda (Fig. 1). While the new crown rust resistant derivatives from Victoria (Boone, Tama, Control, Cedar, Vicland, Vikota, Osage, Neosho, Ventura, Forvic, Letoria, Victorgrain, Fulgrain, Stanton and others) were being developed and released, the helminthosporium blight was unknown and apparently of little or no importance as these varieties were consistently high in yield because of their relative freedom from disease, particularly crown rust. With the sudden appearance of this new disease in the field in 1945, however, and its rapid development to epiphytotic proportions on the Victoria derivatives in certain areas in 1946, the need for further study became imperative.

Although there were available certain methods for inoculating oats to determine their susceptibility to *Helminthosporium victoriae* in the greenhouse, they were not feasible for large scale use in the field. The relative effectiveness of several techniques applicable to large-scale evaluation in

the greenhouse and in the field were therefore compared and the results are herein reported.

MATERIALS AND METHODS

During early spring and autumn of 1946, investigations were conducted in the greenhouse and in nursery at the Agronomy Farm at Ames, Iowa, to determine the relative effectiveness of ground, infected straw, naturally infested soil, and macerated cultures of *Helminthosporium victoriae* in inducing infection on susceptible and resistant oat varieties.

The infected straw was of susceptible varieties grown in nursery experiments either at the Agronomy Farm or at the Ash Avenue Farm at Ames,
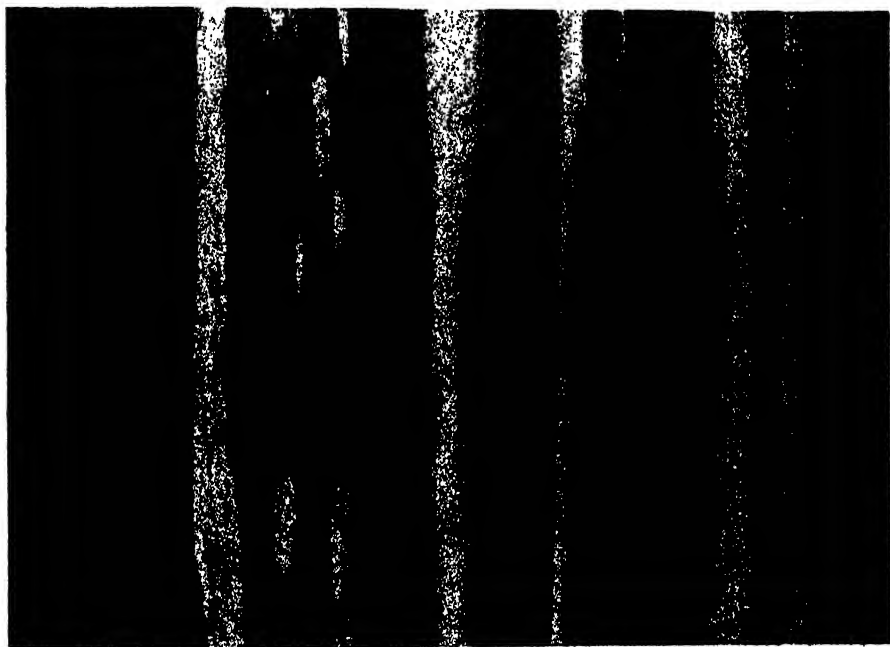
FIG. 1.   Victoria oat leaves showing: 1, check; 2 and 3, ''Victoria type'' resistance to crown rust, the latter showing individual points of infection; and 4 to 7, characteristic striping from helminthosporium blight.

Iowa. The straw from the Agronomy Farm was naturally infected, while that of the Tama plants at the Ash Avenue Farm had been artificially inoculated with a spray of mycelial suspension just before jointing, during a cloudy, rainy period. The darkened nodes and stems of the straw, which contained abundant conidia of the fungus, were ground in a hammermill to a size that would pass through openings about 1 mm. in diameter. In all the trials involving ground straw one teaspoonful of such material was added to each 4-inch pot or 5-foot nursery row. The naturally infested soil was from the Agronomy Farm where badly infected oats had been grown the previous crop season.

For the experiments with artificial cultures of *Helminthosporium vic-*

toriae, the following three cultures were used: (1) The original isolate (H96) and (2) a sporulating strain (H224), both isolated from oats at Ames, Iowa; and (3) an isolate from oats (designated H.R.R.) received from Dr. H. R. Rosen, Fayetteville, Arkansas. These cultures were grown on oatmeal agar in 100 × 15 mm. Petri dishes, each containing about 15 cc. agar, for at least ten days at room temperature, and then macerated for 45 seconds in a Waring Blendor. Ten Petri-dish cultures, with or without the agar, that is, in the latter, the culture scraped from the agar, were added to 500 cc. distilled water in the blender. This, when macerated, constituted the "basic suspension." This basic suspension (designated B.S.) and also dilutions of it, one part basic suspension to one part distilled water (designated "50 per cent B.S.") or one part basic suspension to 19 parts distilled water (designated "5 per cent B.S."), were used as inoculum. Unless

Fig. 2. Relative susceptibility of: 1, Victoria, 2, Vicland, and 3, Bond, when sprayed with *Helminthosporium victoriae*, basic suspension without agar.

otherwise stated, these suspensions were applied at the rate of 50 cc. for each 4-inch pot. When the plants were sprayed with any suspension, the leaves were previously rubbed gently to remove most of the waxy covering.

In the greenhouse experiments, 25 to 50 seeds were sown in each 4-inch pot, while in the field experiments, 100 seeds were planted in each 5-foot nursery row. All treatments were replicated three times except in one experiment where the resistant variety was replicated only once. The experimental design of all tests was a randomized block.

Except where naturally infested soil was used, steamed soil, consisting of equal parts of compost, sand, and Clarion loam, was used in the greenhouse experiments. Temperatures in the greenhouse for the duration of most experiments ranged from 65° to 90° F., except in the temperature-controlled experiments.

RESULTS OF GREENHOUSE EXPERIMENTS

After preliminary tests had been made in the late winter of 1946 on several different methods of inducing infection of oats with *Helmintho-sporium victoriae*, seven series of experiments were conducted in the greenhouse at 50° F. and 80° F. with the most promising methods. These included the use of ground infected straw and stubble, infested soil, and the fungus grown artificially on oatmeal agar, and handled in various ways. Some of the typical data on emergence and survival are given in table 1 and illustrated in figures 2 and 3.

No plants of susceptible varieties survived when the basic suspension of either dilution, either with or without the agar, was used as inoculum. The treatments were equally effective when the suspension was added to the seed at sowing time or applied as a spray on the young oat seedlings and incubated in a moist chamber for 36 hours. Usually comparable results were obtained from the basic suspensions and the 50 per cent and the 5 per cent dilutions from them, respectively, whether they were applied with the seed at sowing or as a spray on the seedlings.

No significant differences were observed between the use of cultures removed from the agar and those used together with the agar in preparing the basic suspensions. Both methods were effective in eliminating all plants of susceptible varieties. The basic suspension with the agar, applied directly to the seed, dried, and stored at room temperature for various periods up to more than four months also was effective. As will be pointed out in the following section, this method affords an excellent means of handling oat material for field or nursery plantings.

When ground infected straw was used as a source of inoculum (Series A, 2 and 3) a few of the susceptible plants escaped being killed when the straw was added to the seed at the time of sowing. These surviving plants were considerably etiolated and less than one-half the height of plants grown in sterile soil. It is doubtful that these plants would have produced seed if allowed to continue development. When the infected straw was applied broadcast on previously rubbed and wetted plants, however, or applied to the surface of the soil after the oat plants had emerged and the potted plants placed in the moist chamber for 36 hours, the infected straw was as effective as the basic suspension for obtaining complete infection in susceptible varieties. When infected straw is used as a source of inoculum, apparently incubation in the moist chamber is necessary for complete elimination of the susceptible plants. Otherwise escapes may occur.

Soil infested with *Helminthosporium victoriae* did not prove entirely satisfactory for inducing uniform infection in susceptible varieties in the greenhouse, as some plants escaped attack (Series A, 9–10). Where sharp differences between resistance and susceptibility are required, this method is not so satisfactory as the others that eliminate the susceptible plants completely.
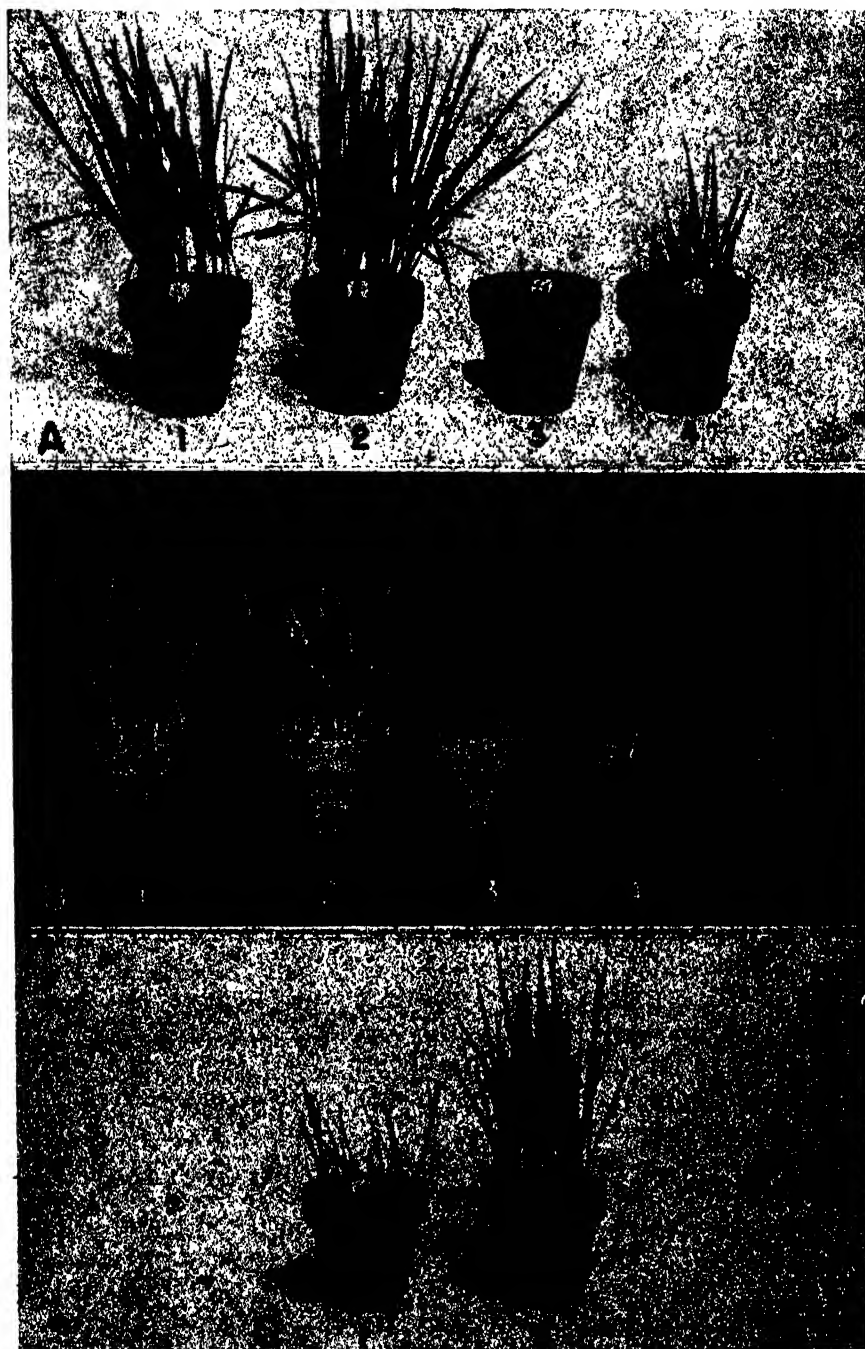
FIG. 3. Clinton, Vicland, and Cedar oat plants grown in the greenhouse from seed noninoculated or variously inoculated with *Helminthosporium victoriae* at sowing or

All emerged plants of resistant varieties continued to develop normally while infected plants òf susceptible varieties rarely developed beyond the primary leaf stage. The emergence of susceptible varieties varied, with the method of inoculation employed, from zero to nearly 100 per cent. The lowest emergence counts were obtained at the higher temperature (80° F.). especially when the seed had been inoculated with basic or 5 per cent basic suspension (Series A, 15 and 16; Series F, 1 and 2). The lower temperature (50° F.) was somewhat less favorable for the fungus than the higher temperature and consequently percentage emergence usually was lower at the higher temperature. At both temperatures, however, the percentage survival was uniformly zero. With the resistant variety, Clinton, emergence was depressed comparatively little by the inoculation. In only one case (Series A, 15), was it depressed to as low as 64 per cent. In this case the basic suspension had been used with the seed at sowing. Shallower seedings and the use of more dilute suspension should overcome any significant emergence depression that may otherwise occur in resistant varieties.

In some cases (Series E, 4) the use of the macerated sterile oatmeal-agar alone delayed germination of both susceptible and resistant oat varieties as well as reduced emergence. In other cases (Series F, 5) no significant effects were apparent.

In no case did the resistant varieties included in this test succumb to or show any symptoms of being infected by *Helminthosporium victoriae*. Seed that failed to germinate or seedlings that failed to emerge showed no evidence of infection. Only those varieties that have the crown rust resistance of Victoria were found susceptible. In fact, this was one of the methods used to determine whether surviving plants were escapes or mixtures. The surviving plants were inoculated with crown rust race 45. If susceptible they were classed as mixtures and if having the Victoria type of crown rust resistance, they were classed as escapes. The final check for determining escapes, however, was by spraying the plants with a mycelial suspension of *H. victoriae*.

The above results indicated that resistance or susceptibility of oats to *Helminthosporium victoriae* could be accurately and readily determined in the greenhouse by inoculating with infested straw or especially with suspensions of cultures of the fungus either with or without the agar.

before, when so stated: A, 1, Clinton inoculated with culture H96, basic suspension (=B.S.), with agar; A, 2, Clinton inoculated with H96, 5 per cent B.S., with agar; A, 3, Vicland inoculated with H96, B.S., with agar; A, 4, Vicland inoculated with H96, 5 per cent B.S., with agar; B, 1, Clinton not inoculated (control); B, 2, Vicland not inoculated (control); B, 3, Vicland inoculated with H224, 5 per cent B.S., with agar; B, 4, Vicland inoculated with H96, 5 per cent B.S., with agar; B, 5, Vicland, inoculated with culture H.R.R. from Arkansas, 5 per cent B.S., with agar; C, 1, Cedar, seed moistened with B.S. of H96, incubated 20 hours at room temperature and stored 4 months before sowing; C, 2, Cedar not inoculated (control).

TABLE 1.—*Emergence and survival of Clinton (Resistant) and Victoria, Vicland, and Cedar (Susceptible) oats following various methods of inoculation with Helminthosporium victoriae in the greenhouse at 50° and 80° F., Ames, Iowa, 1946*

Series and unit No.	Inoculum[a] and method[b] of inoculation	Resistant variety		Susceptible variety			
		50° F.	80° F.	50° F.		80° F.	
		Emerg.[c]	Emerg.[c]	Emerg.	Surv.	Emerg.	Surv.
		Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
		Clinton		*Victoria*			
A, 1	None (Control)		96	89	100	83	100
						Vicland	
D, 7	Do	98	90	100	100	96	100
, 8	None (Seed treated)[d]	100	100	100	100	100	100
E, 4	With sterile agar, with seed at sowing[e]		95			85	100
, 5	None (Control)		100			99	100
F, 5	With sterile agar, with seed at sowing[e]	98	100				
, 6	None (Control)	100	97				
, 7	None (Seed treated)[d]	100	100				
	Culture unidentified					*Victoria*	
A, 2	Ground straw, with seed at sowing		88	64	0	57	4
, 3	Ground stubble,　　do		88	80	7	79	13
, 4	Ground straw, on soil surface when plants emerged		96			96	0
, 5	Ground stubble,　　do		92			91	3
, 6	Ground straw, on emerged plants		96			94	0
, 7	Ground stubble,　　do		92			92	0
, 8	Ground straw, on soil surface at sowing		88			87	0
, 9	Infested soil I		88	80	0	83	2
, 10	Infested soil II		96	75	8	79	8
	Culture H96 (Basic suspension)						
A, 11	Without agar, sprayed on seedlings		92			88	0
, 13	With agar,　　do		92			89	0
, 15	Without agar, with seed at sowing		64		0	3	0
						Vicland	
E, 3	Do		96				
F, 1	Do	93	87	15	0	12	0
D, 2	With agar, with seed at sowing	86	96	50	0	10	0
E, 2	Do		73			50	0
F, 3	Do		82			0	0
						Victoria	
A, 17	Do		80		0	21	0
						Cedar	
B, 1	With agar, on seed, stored 0 days					40	0
C, 1	Do　　　60					64	0
G, 1	Do　　　90					68	0
D, 1	Do　　　120					72	0
E, 1	Do　　　130					44	0

<div align="center">TABLE 1—<i>(Continued)</i></div>

Series and unit No.	Inoculum[a] and method[b] of inoculation	Resistant variety 50° F. Emerg.[c]	Resistant variety 80° F. Emerg.[c]	Susceptible variety 50° F. Emerg.	Susceptible variety 50° F. Surv.	Susceptible variety 80° F. Emerg.	Susceptible variety 80° F. Surv.
		Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
	(50 per cent basic suspension)		*Clinton*			*Vicland*	
D, 5	Without agar, with seed at sowing	100	94	76	0	36	0
, 3	With agar, do	96	96	68	0	26	0
	(5 per cent basic suspension)					*Victoria*	
A, 12	Without agar, sprayed on seedlings	. . .	92	. . ..	... ..	88	0
, 14	With agar, do	... ...	96	.. ..	....	88	0
, 16	Without agar, with seed at sowing	....	76	....	0	8	0
						Vicland	
D, 6	Do	100	98	92	. 0	68	0
F, 2	Do	100	96	40	0	17	0
D, 4	With agar, with seed at sowing	100	99	93	0	82	0
F, 4	Do	....	97	....	....	21	0
						Victoria	
A, 18	Do	.. ....	96	... .	0	32	0
	Culture II.R.R.						
	(50 per cent basic suspension)					*Vicland*	
D, 9	Without agar, with seed at sowing	100	96	66	0	46	0
	(5 per cent basic suspension)						
D, 10	Without agar, with seed at sowing	100	100	84	0	92	0
	Culture H224						
	(Basic suspension)						
C, 2	Without agar, sprayed on seedlings	.. ....	....	....	....	....	0
	(50 per cent basic suspension)						
D, 11	Without agar, with seed at sowing	100	98	100	0	86	0
, 13	With agar, do	100	98	86	0	78	0
	(5 per cent basic strength)						
D, 12	Without agar, with seed at sowing	100	100	92	0	90	0
, 14	With agar, do	98	100	92	0	84	0

[a] Ground straw inoculum was from Ash Avenue Farm; ground stubble, from Agronomy Farm. Infested soils I and II were from Agronomy Farm.

[b] When straw or stubble inoculum was placed on surface of the soil after plants emerged, or on emerged plants, the plants were placed in a moist chamber for 36 hours after inoculation. When seed was inoculated and stored before planting, the seed was moistened with the basic suspension, incubated 20 hours, then dried and stored.

[c] In all cases 100 per cent of the resistant Clinton plants survived.

[d] Seed in Series D, 8 and F, 7 was treated with DuBay 1452–F (6.5 per cent ethyl mercuri-p-toluene sulfonanilide).

[e] The sterile oatmeal agar was applied at the same rate as the treatment with viable basic suspension.

RESULTS OF NURSERY EXPERIMENTS

Since the greenhouse experiments had indicated that some of the methods of inoculation might be satisfactory for use in the field, six methods of inoculation, four with infected straw and two with basic suspension, together with a noninoculated control, were included in an experiment in the field on summer fallow. Although seeded in early September, 1946, the weather that followed was comparable to that usually prevailing in the spring season. The resulting data on survival are given in table 2.

TABLE 2.—*Percentage survival of plants of susceptible[a] Vicland and Cedar oats following different methods of inoculation with Helminthosporium victoriae in the field at Ames, Iowa, 1946*

Treatment No.	Inoculum and method of inoculation	Percentage plants surviving			
		Vicland	Cedar		Ave.
		I	I	II	
1	None (Control)	100	100	100	100
2	Ground straw, on soil surface	6	2	46	18
3	Do , with seed[b]	6	4	20	10
4	Do , do	0	0	6	2
5	Do , moistened on seed and incubated for 20 hours before sowing	2	0	7	3
6	Culture H96, with agar (B.S.[c]), do	0	0	0	0
7	Do , moistened on seed and dried immediately before sowing	0	0	0	0

[a] Resistant Bond and Clinton varieties were also included in the experiment, but no differences in survival were observed with any of the treatments. All plants survived 100 per cent.

[b] Seed was sown ½ inch deep in treatment no. 3 and 1½ inches deep in all of the others.

[c] B. S. indicates basic suspension.
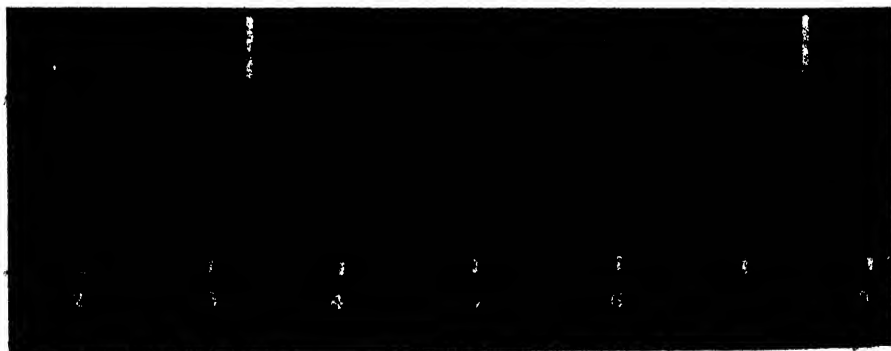
FIG. 4. Representative replication in field nursery comparing methods of inoculating Clinton (rows 1–3) and Cedar (rows 4–8) oats with *Helminthosporium victoriae*. Rows 1 and 7 (= treatment 6, table 2), seed moistened with basic suspension and incubated 20 hours before sowing; rows 2 and 8 (= treatment 2, table 2), ground infected straw on surface of soil after seeding; rows 3 and 5 (= treatment 1, table 2), noninoculated controls; row 4 (= treatment 7, table 2), seed moistened with basic suspension and dried immediately; row 6 (= treatment 4, table 2), seed dusted with ground, infected straw and incubated 20 hours at room temperature before sowing.

The only two methods of inoculation that were uniformly effective in eliminating all plants of susceptible varieties were those used in series 6 (Fig. 4, row 7) and series 7 (Fig. 4, row 4). In both of these, the basic suspension of cultures had been applied to the seed before sowing: Series 6, incubated 20 hours at room temperature, and series 7, dried immediately after inoculation. The results from the use of ground infected straw were erratic, because from 2 to 18 per cent of the susceptible plants escaped the attack of *Helminthosporium victoriae*. Placing the infected straw with the seed at sowing time at a depth of 1¼ inches was more satisfactory than the shallower seedings.

No loss of plants in the resistant varieties was apparent, indicating that the more effective methods can be satisfactorily used under nursery or field conditions to determine varietal reaction to this disease.

SUMMARY

Methods of inoculating oats with *Helminthosporium victoriae* under greenhouse and nursery conditions were compared at Ames, Iowa, in 1946. Mycelial suspensions of the organism were found most uniformly satisfactory in inducing heavy infections in susceptible varieties of oats. The basic suspensions and also 50 per cent and 5 per cent dilutions of them, with or without the agar, applied with the seed at sowing, on the surface of the soil after emergence, or as a spray on the seedlings after emergence, gave no survival in susceptible varieties, while corresponding plants of resistant Clinton were not significantly injured. When the suspensions were applied on the surface of the soil after emergence or as a spray on the plants, incubation in a moist chamber for 36 hours was necessary.

Seed moistened with the basic suspension, dried immediately or incubated at room temperature for 20 hours, planted immediately or stored for various periods up to more than 4 months also gave equally satisfactory results in both greenhouse and nursery experiments.

In the greenhouse, when *Helminthosporium*-infected straw was applied broadcast to the soil surface or on moistened emerged plants at the rate of one teaspoonful per pot and placed in the moist chamber for 36 hours no survival of susceptible plants was obtained. Dusting the seed with infested straw at seeding resulted in some susceptible plants surviving with every inoculation in the field and in the greenhouse experiments.

When sharp differences are necessary in a critical study of resistance or susceptibility of oats to this fungus, the method in which suspensions of cultures is used is preferred. Where large numbers of oat varieties or selections are to be tested under field conditions, however, and less critical differences are satisfactory, ground infected straw sown with the seed may be used.

In susceptible varieties emergence was reduced as much as 100 per cent by some methods of inoculation. In resistant varieties a slight depression

in emergence was observed, but all emerged plants continued to develop normally while infected plants of susceptible varieties rarely developed beyond the primary leaf stage. Germinated seeds of resistant varieties which did not emerge showed no evidence of infection. Percentage survival, therefore, is the best criterion for rating relative resistance of oat varieties to *Helminthosporium victoriae*.

AGRICULTURAL EXPERIMENT STATION,
   AMES, IOWA.

TREATMENT OF LILY BULBS FOR BLACK SCALE CONTROL

F . J . L e B e a u a n d F . J . R e y n o l d s

Black scale of Easter lily (*Lilium longiflorum*) was described by Plakidas[1] as causing severe losses to lily growers in Plaquemines Parish, Louisiana, as early as 1937. The disease increased and by 1943 there remained very few stocks of clean bulbs and few non-infested fields in the lily growing area. The increased value of lily bulbs arising from the curtailment of importation of this plant in 1941–42 stimulated a rapid expansion of lily bulb production in Terrebonne Parish, Louisiana, an area which had formerly produced few commercial bulbs. In the face of strong demand for large amounts of seed stock, little attention was paid to diseases, with the result that black scale as well as the virus diseases were introduced on the seed stock and widely distributed in Terrebonne Parish. Black scale became so threatening that in 1943, at the request of the lily growers in Terrebonne Parish, a rigid quarantine controlling the use of diseased stock was set up by the Louisiana State Department of Agriculture. While these measures reduced to some extent the further spread of the disease, black scale had become so well distributed before the quarantine that more adequate control measures were needed.

In preliminary bulb-treatment tests, Plakidas[1] used the common disinfectants available at that time. No one of these destroyed the pathogen in and on diseased bulbs or prevented infection of clean bulbs in the soil. Many of the fungicidal treatments were definitely injurious to the bulbs. In 1944, the senior writer took over the investigations and tested the more recently available organic fungicides.[2,3] The present paper reports the results of tests with a number of fungicides for controlling bulb-borne infection of *Colletotrichum lilii* and presents additional information on the use of Puratized N5E (phenyl mercuric triethanol-ammonium-lactate) for controlling black scale.

EXPERIMENTAL RESULTS

Most evaluations of the fungicides have been based on a laboratory test[2] which has given results that have agreed closely with those obtained in greenhouse and field tests. This test consisted of soaking detached infected scales in a fungicide for 24–48 hours and then soaking them in sterile water for 24 hours. After a 5-min. treatment in a saturated calcium hypochlorite solution, the scales were plated on potato-dextrose agar. In the more recent

---

[1] Plakidas, A. G. Black Scale: A disease of Easter Lily bulbs. Phytopath. **34**: 556–571. 1944.

[2] LeBeau, F. J. The eradicant action of a fungicide on *Colletotrichum lilii* in lily bulbs. Phytopath. **36**: 391–393. 1946.

[3] ——————. A fungicide for protecting lily bulbs from infection by *Colletotrichum lilii*. Phytopath. **37**: 194–196. 1947.

work a wash in running tap water for 3–4 hours replaced the 24-hour soak in sterile water. Fungicide treatments were considered promising only if they eliminated *Colletotrichum lilii* and greatly reduced the other fungi associated with the *Colletotrichum*. When a treatment was ineffective *C. lilii* grew out from plated diseased scales and, in addition, a number of other fungi, principally species of *Fusarium* and *Penicillium,* appeared in the plates. Frequently the other fungi exceeded the *Colletotrichum*. Practically all lots of control scales, scales bearing black scale lesions and plated after a 5-min. treatment in calcium hypochlorite solution, have given rise to fungi. The fungus *Colletotrichum lilii* has appeared in approximately 50 per cent of the control scales, the incidence varying from 10 to 75 per cent in different test lots. Plakidas[1] noted that the ease of isolating *C. lilii* from diseased tissue varied from the extreme cases in which few cultures of *C. lilii* could be obtained from positively infected material to those in which 50 per cent or more of tissue platings yielded the pathogen. The ease with which *C. lilii* could be isolated varied with storage time after harvest, isolation being more difficult as storage time increased. In the present work scales stored for periods of 1–2 weeks to several months were used. In table 1, data on tests involving several materials are presented. Others tested, as Puratized 804 and IN 1452F (ethyl mercuric p-toluene sulfoanalide) at dilutions up to 1–4000, Dowicide 9B (zinc trichlorophenate) and New Im-

TABLE 1.—*The effects of fungicides on the survival of Colletotrichum lilii in in-fected lily scales*

Materials[a]	Concentration[b]	Time (Hours)	Number of trials	Number of scales treated	Number of scales with	
					Total fungi[c]	C. lilii
Puratized N5E	1–1000	24	....	150	0	0
Do (without wetting agent)	1–1000	24	......	110	0	0
Puratized N5E	1–2000	48	...	70	0	0
Do	1–8000	48	2	22	2	0
Puratized M. C.	1–1000	24	2	16	5	5
Isothan Q15	1–500	24	1	11	6	3
	1–1000	24	1	10	5	1
Dithane 14	1–100	24	1	9	....	3
	1–1000	24	1	9	....	4
8-Hydroxyquinoline sulphate	1–500	24	1	13	....	5
Formalin, 5 per cent at 45° C.	....	1	1	9	6	2
Control	....	......	......	176	170	85

[a] Except where otherwise noted, IN 181P, a sodium lauryl wetting agent, was added at the rate of 1–4000. The Puratized compounds are phenyl mercuric triethanol-ammonium lactate; Isothan Q15 is lauryl isoquinolinium bromide; and Dithane 14 is a liquid disodium ethylene bisdithiocarbamate.

[b] Concentration based on the commercial product or experimental sample used and not on active ingredients. In all cases an excess of solution over that needed to cover the scales was used.

[c] Species of *Fusarium* and *Penicillium* were the predominant fungi appearing from plated scales.

proved Ceresan (ethyl mercuric phosphate) at dilutions up to 1–5000, and Dowicide A and B at dilutions up to 1–1000, were not included since injury to the scales was too severe for them to have any usefulness.

Only one material, Puratized N5E,[4] effectively destroyed all fungi occurring in infected scales without causing severe injury to healthy scales. When diseased lily scales were exposed to Puratized N5E, the infected areas were freed from all fungi and some of the healthy tissue surrounding the lesions was killed by the fungicide although healthy portions of the lily scale more distant from lesions were unaffected. When healthy lily scales were treated, the basal portions of the scales where they were broken from the bulbs were killed. Injury was also evident at points of other mechanical injury.

A wide range of concentrations of Puratized N5E was effective, depending upon the duration of treatment and the absolute amount of the toxicant in relation to the amount of plant material being treated. These relationships are illustrated by the data in table 2. A study of table 2 reveals that: (1) Regardless of concentration, a minimum time (about 24 hours) was required to completely eliminate *Colletotrichum lilii* from infected scales; (2) given the minimum effective time of treatment, an additional time, as was required for the more dilute solutions, merely served to compensate for the greater dilution rather than for the reduction in absolute amounts of the toxicant, since the combination of low concentration and long treatment was effective only when an excess amount of solution, in relation to the amount of scales being treated, was used; (3) in the range of concentration used, a second use of the same solution was effective only when an excess of solution over that required to cover the scales was used in the first treatment; (4) the most satisfactory combination of time and concentration from a practical standpoint is probably that of 24-hour treatment with a 1–1000 solution of Puratized N5E.

Other Factors Affecting the Use of Puratized N5E for Black Scale Control

Since it appeared that the long time needed for eradicating *Colletotrichum lilii* from diseased scales by Puratized N5E was required in order that the toxicant could penetrate the diseased tissues, the influence of reduced pressure on the effectiveness of the treatment was investigated. Infected scales in 1–500 and 1–1000 solutions of Puratized N5E were subjected to reduced pressure by the continuous operation of a vacuum pump, with an efficiency rating of 0.1 micron of mercury, for periods of 15 and 30 minutes, after which normal pressure was reestablished and the scales allowed to remain in the treating solution for such a time as to make the total time in the treating solution 1, 6, 12, and 24 hours. In no case was the 1-hour, and rarely was the 6-hour, treatment effective. In three sets of experiments

[4] Here Puratized N5E is used to include also Puratized N5X and Puratized Agricultural Spray, since each of these materials contains the same toxicant and when appropriately diluted has given similar results. The bulk of the data secured was with Puratized N5E.

involving 60 scales each, the 12-hour and the 24-hour treatments including either 15 or 30 minutes under reduced pressure completely eliminated all fungi. The use of reduced pressure was attended by greater injury than treatment at normal pressures. In one field test the reduction of pressure for 15 minutes with 6 hours total treating time (Table 5) caused a reduction in emergence. Only a fair degree of control was obtained.

TABLE 2.—*Relation of time, concentration, volume of treating solution in relation to volume of plant material, and successive use of the same solution on the effectiveness of Puratized N5E in eradicating Colletotrichum lilii from infected lily scales*

Concentration of Puratized N5E	Time (Hours)	Volume of solution	Times used	Number of scales plated	Number of scales with	
					Total fungi	*C. lilii*
1–500	6	excess[b]	1st	20	1	1
1–500	12	excess	do	20	1	1
⎰ 1–500[a]	12	no excess[c]	do	30	0	0
⎱ 1–500	24	excess	2nd	20	0	0
⎰ 1–500	24	excess	1st	15	0	0
⎱ 1–500	24	excess	2nd	15	0	0
⎰ 1–500	24	no excess	1st	36	0	0
⎱ 1–500	24	excess	2nd	20	4	2
1–1000	6	excess	1st	32	2	1
1–1000	12	excess	do	60	0	0
⎰ 1–1000	12	no excess	do	32	3	1
⎱ 1–1000	36	excess	2nd	26	3	2
⎰ 1–1000	24	excess	1st	40	0	0
⎱ 1–1000	24	excess	2nd	40	0	0
⎰ 1–1000	24	no excess	1st	50	0	0
⎱ 1–1000	24	excess	2nd	43	18	4
1–4000	12	excess	1st	40	17	7
⎰ 1–4000	24	excess	do	40	1	0
⎱ 1–4000	24	excess	2nd	20	2	0
⎰ 1–4000	24	no excess	1st	55	4	3
⎱ 1–4000	24	excess	2nd	45	28	15
1–4000	48	excess	1st	34	0	0
1–8000	24	excess	do	10	3	3
⎰ 1–8000	24	no excess	do	30	6	6
⎱ 1–8000	24	excess	2nd	26	21	9
1–8000	48	no excess	1st	36	12	4
1–8000	48	excess	do	22	2	0

[a] Indicates successive use of the same solution.
[b] An excess of solution was used over that required to cover the scales.
[c] Only enough solution to cover the scales was used.

Puratized N5E does not protect treated bulbs from reinfection when exposed to inoculum in infested soil, but, if after the Puratized N5E treatment, Arasan is dusted on the bulbs, both bulb-borne and soil-borne infections are controlled. Since the simultaneous application of both treatments would have a practical advantage, the effects of the presence of Arasan (tetramethyl-thiuram-disulfide), Tersan (similar to Arasan, but with a wetting agent), Fermate (ferric dimethyldithiocarbamate), Phygon (2,3,di-

chlor-1,4-naphthoquinone), and Yellow Cuprocide (yellow cuprous oxide) on the eradicant action of Puratized N5E were tested using the laboratory method of evaluation. These protective fungicides were added to 1–500 and 1–1000 solutions of Puratized N5E at the rate of one per cent of the protective fungicide and the suspensions obtained were used for treating infected lily scales. After treatment the scales were thoroughly washed in running tap water for several hours and after a 5-minute wash in calcium hypochlorite solution they were plated on potato-dextrose agar. The results of the test are shown in table 3.

TABLE 3.—*The effects of other fungicides added to Puratized N5E solutions on the eradicant action of Puratized N5E towards Colletotrichum lilii*

Concentration of Puratized N5E	Fungicide added	Number of scales treated	Number of scales with	
			Total fungi	*C. lilii*
1–500	Tersan	15	13	4
1–500	Fermate	15	14	3
1–500	Phygon	15	0	0
1–500	Yellow Cuprocide	15	0	0
1–500	None	15	0	0
1–1000	Tersan	15	12	2
1–1000	Fermate	15	15	3
1·1000	Phygon	30	4	0
1–1000	Yellow Cuprocide	15	0	0
1–1000	Arasan	15	12	.....
1–1000	None	30	0	0
None	Tersan	15	14	3
Do	Fermate	15	13	3
Do	Phygon	15	13	5
Do	Yellow Cuprocide	20	20	13
Do	Arasan	20	17	.....
Do	Water control	15	13	3

In these tests all of the fungicides derived from thiocarbamic acid interfered with the action of Puratized N5E when used simultaneously. Yellow Cuprocide had no effect while Phygon probably had only a slight inhibiting effect. None of the protective fungicides alone reduced to any appreciable extent the number of scales bearing *Colletotrichum lilii* or other fungi. Yellow Cuprocide seemed to have enhanced the ease with which *C. lilii* grew out from the scales.

Dupont IN 181P and Dupont IN 3622, detergents based upon sodium lauryl sulphate, were used as wetting agents in the Puratized N5E solutions, but no difference in the action of Puratized N5E was noted. These agents were used at the rate of 1–4000 in solutions of Puratized N5E varying in concentration from 1–500 to 1–4000 for time intervals of 12 to 48 hours.

Field and Greenhouse Studies

Only a few materials were tried in the greenhouse, and only the Puratized compounds were tested in the field. In the greenhouse tests, stem bulblets from a badly infected stock were treated and after treatment

planted in steamed Yohola silt loam which is the major soil type of the commercial lily area. Seven to eight months after planting, the bulbs were harvested for observation. Typical data from these tests are presented in table 4. Injury, evidenced by retarded and reduced emergence, was produced by treatments 1, 2, 3, 7, 8. The greatest injury was caused by treatment 1, in which 11 of the 15 bulbs dug had remained dormant throughout the period of the experiment. The New Improved Ceresan treatment retarded emergence as much as 6 weeks and frequently those plants which

TABLE 4.—*The effect of chemical treatment on the development of black scale on lily bulbs in steamed soil in the greenhouse. Bulblets from an infected stock were used*

Test No. and Material	Concentration	Time (Hours)	Number of bulbs harvested	Number of clean bulbs	Number of diseased bulbs in each class[b]			Disease index[c]
					1	2	3	
1 IN 1452F	1–2000	48	15	12	3	0	0	7
2 Dithane	1–100	24	12	6	1	3	2	36
3 Dithane	1–50	24	33	15	7	3	8	38
4 Isothan Q15	1–125	24	13	6	4	1	2	31
5 Isothan Q15	1–250	24	18	11	2	0	5	26
6 Isothan Q15	1–1250	24	19	2	4	3	10	70
7 New Improved Ceresan	1–5000	24	14	9	2	2	1	22
8 New Improved Ceresan	1–5000	48	10	5	4	1	0	20
9 Puratized N5X	1–2000	48	22	22	0	0	0	0
10 do	1–500	24	17	16	1	0	0	2
11 do	1–1000	24	19	18	1	0	0	2
12 do	1–1000	6	19	12	2	1	4	28
13 do	1–2000[a]	48	7	0	2	2	3	71
14 Untreated	....	....	18	3	4	2	9	64

[a] Planted in non-steamed badly infested soil from Terrebonne Parish.
[b] 1 = Mildly diseased; 2 = moderately diseased; 3 = severely diseased.
[c] Clean, mild, moderately and severely diseased bulbs were given numerical values of 0, 33.3, 66.6 and 100 respectively. The disease index for any given treatment was obtained by summing the products of the number of bulbs in each class and the numerical value of the classes and dividing by the total number of bulbs.

emerged were malformed. The bulbs, however, were not destroyed, but persisted to the end of the test. While both New Improved Ceresan and IN 1452F reduced the incidence of black scale, both materials were inferior to Puratized N5X.

Puratized N5X almost completely prevented the development of black scale in all these tests when concentrations of 1–1000 to 1–2000 were used in 24- and 48-hour treatments. That Puratized N5X had no protective value is indicated by treatment 13.

The results of field tests on the use of Puratized N5X and N5E are given in table 5. Tests 2, 3, 4 were with commercial seed stock in which there was a high percentage of infected bulbs, the infection varying from mild to severe for individual bulbs. The over-all degree of infection was indicated as mild, moderate, and moderate for tests 2, 3, and 4, respectively. Tests

1 and 5 were with bulbs selected because of a high percentage of mild to moderate infection. In each case the bulbs were planted in non-infested soil. These tests covered two growing seasons (1944–45 and 1945–46) and gave results typical of those obtained by the majority of the growers who used Puratized N5E on the 1945–46 crop.

These results agree well with the laboratory and greenhouse data. A 1–4000 solution of Puratized N5X or N5E was not so effective in the field test as in many of the laboratory tests. In the field tests only enough solution was used to cover the bulbs. When a 1–4000 solution was used in this manner in the laboratory (Table 2) control was likewise poor. A 1–4000

TABLE 5.—*The effects of Puratized N5X and N5E on the development of black scale on previously diseased bulbs treated and planted in clean soil in the field*

Test No. and Material	Concentration	Time (Hours)	Number of bulbs harvested	Number of clean bulbs	Number of diseased bulbs in each class[b]			Disease index[c]
					1	2	3	
1 Puratized N5X	1–1000	48	98	96	2	0	0	1
	1–4000	48	96	42	17	10	27	41
	1–1000[a]	6	36	27	3	1	5	19
Check	.. ..	....	91	17	4	7	63	74
2 Puratized N5X	1–2000	48	300	258	42	0	0	4
Check	.. ..	.	318	58	240	18	2	31
3 Puratized N5E	1–2000	48	600	562	13	16	9	4
Check	.. ..	..	736	442	94	84	106	26
4 Puratized N5X	1–1000	24	300	272	27	1	0	3
	1–2000	24	300	277	12	9	2	4
	1–4000	24	300	216	53	9	22	15
5 Puratized N5E	1–1000	24	100	91	5	3	1	5
	1–2000	24	93	85	6	2	0	4
	1–4000	24	98	32	40	15	11	35
Check	... ...	.... ..	94	20	12	12	50	66

[a] Reduced pressure for 15 min.

[b] 1 = Mildly diseased; 2 = moderately diseased; 3 = severely diseased.

[c] Clean, mild, moderately and severely diseased bulbs were given numerical values of 0, 33.3, 66.6 and 100 respectively. The disease index for any given treatment was obtained by summing the products of the number of bulbs in each class and the numerical value of the classes and dividing by the total number of bulbs.

solution is therefore inadequate for practical use. There seems to be little choice between a 1–1000 solution or a 1–2000 solution except that a 1–2000 concentration may be nearing the point of non-effectiveness. A treatment time in excess of 24 hours was of little value in field tests conducted on as nearly a practical scale as was possible.

SUMMARY

Several fungicides were tested for control of black scale of Easter-lilies. Only one, phenyl mercuric triethanol-ammonium-lactate, known under the code and trade names of Puratized N5X, Puratized N5E, or Puratized Agricultural Spray, was effective in eliminating *Colletotrichum lilii* from in-

fected lily-bulb tissues in laboratory tests without severe injury to the host tissue. At concentrations of 1–1000 to 1–2000 of the 10 per cent preparation, and with treatment for 24 hr., this fungicide gave practical control of bulb-borne infection in the field in two years, 1944–45 and 1945–46.

The eradicant action of Puratized N5E was inhibited by fungicides derived from thiocarbamic acid.

DEPARTMENT OF PLANT PATHOLOGY,
LOUISIANA AGRICULTURAL EXPERIMENT STATION,
BATON ROUGE, LOUISIANA.

A SEED–BORNE VIRUS OF MUSKMELON

WM. E. RADER,[1] HUGH F. FITZPATRICK,[2]
AND E. M. HILDEBRAND[3]

A mosaic disease of muskmelon caused by a seed-borne virus has been observed in New York plantings since 1936. In attempting to establish the identity of the causal virus it was found that transmission by mechanical means was readily achieved from melon to melon but not from melon to Turkish tobacco. Because of this it was suspected that the mosaic was not caused by the cucumber-mosaic virus (*Marmor cucumeris* var. *vulgare* Holmes). In 1941, experiments comparing an authentic cucumber-mosaic virus 1, a mild strain of cucumber-mosaic virus from Indiana, and the melon-mosaic virus on melon, cucumber, and tobacco made it apparent that the melon virus present in New York was unlike the cucumber virus.

The seed-borne muskmelon mosaic virus disease does not cause serious losses in New York judging by its known distribution and its relatively mild to moderate effects on the plants. The relationship of the virus under discussion to those on muskmelons in California and Michigan are not known.

The first report on a seed-borne virosis of muskmelons was made by Kendrick (4) in California who states that out of a total of 1,519 plants grown in the greenhouse from 23 packets of seed, only 27, or 0.23 per cent showed leaf symptoms within two weeks from planting. One hundred per cent infection was obtained by him in artificial inoculations on several varieties of muskmelon and summer squash, and symptoms comparable to those on Honey Dew muskmelon were obtained in 10 to 21 days after inoculation. The symptoms usually were severe, the leaves becoming reduced in size and modified in shape.

Mahoney (5) observed a seed-borne mosaic virus in inbred lines of muskmelon in Michigan. Infected plants showed typical mosaic on the first true leaf; their growth was slow in the field and setting of the fruit was delayed from 10 to 14 days. The mosaic was observed to spread rapidly through the field, so that by September 14 nearly all the plants were diseased. An average infection of 24 per cent was found in 6 out of 48 inbred progenies which showed mosaic. Further selections of these progenies always showed typical mosaic symptoms.

Middleton (6) describes a squash-mosaic virus disease which is seed transmitted. According to Freitag (in correspondence) this is the same

[1] Plant Pathologist, Shell Agricultural Laboratory, Modesto, California. Formerly assistant, Department of Plant Pathology, Cornell University, Ithaca, New York.
[2] Plant Pathologist, Food Machinery Corporation, Riverside, California. Formerly assistant, Department of Plant Pathology, Cornell University, Ithaca, New York.
[3] Professor of Plant Pathology, Texas A. and M. College, College Station, Texas. Formerly Assistant Professor, Department of Plant Pathology, Cornell University, Ithaca, New York.

virus which Kendrick (4) studied in muskmelons. Freitag believes that the disease is caused by a different virus from that encountered by Mahoney (5) because of the much higher percentage of seed transmission that Mahoney found.

Fields (1) records a squash-mosaic virus (not *Marmor cucumeris* var. *vulgare* II.) from California. He states (on the authority of Drs. H. H. P. Severin and J. H. Freitag) that the mosaic produced by this virus is the second prevalent squash disease in California.

SYMPTOMATOLOGY

In New York, symptoms appear on the first leaves of seedling musk-melons about two weeks after planting in the greenhouse (Fig. 1). Several

FIG. 1. New York muskmelon mosaic. Fourteen-day-old diseased muskmelon seedling, variety Queen of Colorado, showing veinbanding, distortion, and characteristic serration of the leaves.

different leaf symptoms may appear on the same plant (Fig. 2). The first leaf to show symptoms usually has a conspicuous veinbanding consisting of a narrow border of dark green parallel with each of the main veins of the leaf, the remainder of the leaf remaining the usual lighter green. The leaves which are formed later with rare exceptions do not show veinbanding, but are mottled without reference to the veins (Fig. 2, A). The leaves of the highly susceptible variety, Queen of Colorado, may, however, continue to

show veinbanding. The mottling varies from a coarse pattern of light and dark green through stages in which the leaves are stippled towards the edges with dark green, to cases where the mosaic symptoms are scarcely visible.

A peculiar serration is produced on some foliage in which the tips of

FIG. 2. Diseased muskmelon seedlings, variety Delicious, illustrating the range of symptoms of the New York muskmelon mosaic found on this very susceptible variety: A, seedling showing mottling and abnormal serration of the leaves; B, D, E, leaf distortions found in diseased seedlings; C, veinbanding of the young leaves; F, healthy seedling.

the veins protrude beyond the edge of the leaf, sometimes nearly one-eighth of an inch, giving a toothed appearance (Fig. 2, A and E). Abnormal serration may or may not accompany the mottling. Diseased leaves may also have deformations, which vary from an elongation and a curling in severe cases, to a rather smoothly outlined cordate pattern in mild cases (Fig. 2, B and D). In old infections the cordate leaf pattern may be present in the absence of any marked mottling.

The muskmelon virus gives a typical mosaic pattern in other species and varieties of Cucurbitaceae, although on *Cucumis sativa* L. the mottling is very faint and can easily be overlooked. There are no evident symptoms on the fruits of the susceptible varieties tested except on those of the summer crookneck squash, where they usually show as a green streaking and marbling.

Because of the extreme range of symptoms produced on inoculated seedlings, it was felt at the beginning of this work that there probably were several strains involved. Attempts to segregate strains of the virus by selecting one particular leaf pattern in serial inoculations have failed. Also, there has been no indication of a separation of strains in longevity or thermal inactivation studies.

TRANSMISSION

In studies on melon, 100 per cent transmission was obtained regularly by rubbing leaves of healthy plants with cheesecloth swabs saturated with juice from diseased plants. Symptoms appeared within 10 to 13 days after inoculation. This incubation interval corresponds to that obtained by Kendrick (4) with the California strain. The virus has been recovered from all parts of the plant including roots, stems, leaves, floral parts, and the pollen.

Transmission of mosaic virus through melon seed was observed regularly before the start of this study. Three lots of two-year-old seed which had been taken from three different diseased melons developed virus symptoms on 43, 24, and 12 per cent of the plants. Two lots of seed harvested in 1941 showed 93.5 and 27.9 per cent mosaic. Three years later these same lots of seed gave 2.6 and 6.4 per cent diseased plants.

In limited seed transmission tests in the greenhouse this virus has been transmitted readily through the seeds of the varieties of *Cucumis melo*, *Cucurbita moschata*, *C. flexuosus* and *C. pepo* (Summer crookneck). No seed transmission has been obtained in three separate tests with *Cucumis sativa*.

HOST RANGE STUDIES

All plants used in these experiments were inoculated twice at two-week intervals with carborundum dust being used as an abrasive. Three weeks after the second inoculation juice from these plants was inoculated into healthy muskmelon seedlings in an attempt to recover the virus. Table 1 lists the species of plants from which the muskmelon virus was recovered. All of these plants showed a mottling of the younger leaves with the excep-

tion of cucumber, in which the symptoms were either very faint or at times entirely masked.

TABLE 1.—*Species of plants from which the muskmelon virus was recovered five weeks after inoculation*

Family	Species and varieties[a]
Cucurbitaceae	*Cucurbita maxima* Duchesne (Hubbard, Buttercup, Golden Delicious)
	Cucurbita moschata Duchesne (Long Island Cheese)
	Cucurbita pepo L. (Summer Crookneck, Table Queen, Zuchini)
	Cucumis anguira L. (West India Gherkin)
	Cucumis flexuosus L.
	Cucumis melo L.
	var. *Chito* (Peach-vine or Mango melon)
	var. *conomon*
	var. *inordorus* (Persian melon)
	var. *reticulatus* (Bender, Coopers sweetheart, Davis perfect, Delicious, Honey Rock, Kilgores orange flesh rocky dew, Regular Iroquois, Smith perfect or yellow dew, and 13 × Honey Rock)
	Cucumis sativa L. (National pickle)

[a] Arranged according to the classification proposed by Tapley, Enzie and Van Eseltine (8).

A list of plants which showed no symptoms of the virus following inoculation and from which the virus could not be recovered by the method used in these tests is given in table 2.

Plants showing symptoms of the muskmelon mosaic described here have been readily inoculated with the cucumber 1 virus. The plants thus inoculated subsequently showed a stunting of the leaves and a rosetting of the terminal growths. The diseased progeny from the seed of these plants containing both viruses showed only the typical muskmelon mosaic.

CHARACTERISTICS OF THE VIRUS

The thermal inactivation temperature of the melon-mosaic virus, in expressed sap from diseased plants, is between 60° C. and 62° C. for a 10-minute exposure. Thermal inactivation studies were made by placing 3 cc. of sap expressed from diseased plants in thin-walled vials and lowering into a constant temperature water bath for 10-minute exposures over a temperature range of 54° C. to 74° C. Each test was replicated three times and three separate thermal inactivation tests were made: all gave identical results.

When attempts were made to inactivate the virus in the muskmelon seed with hot water treatments it was found that the thermal death point of the muskmelon seed was approximately the same as that of the virus. It is evident, then, that hot water treatments cannot be expected to destroy the virus within infected seed without killing the melon-embryo.

In all tests the longevity *in vitro* has been more than 74 hours, with a maximum of 250 hours at laboratory temperatures. The sap from plants with mosaic symptoms was infective in all trials after having been dried on

TABLE 2.—*Species of plants from which the muskmelon virus could not be recovered five weeks after inoculation*

Family	Species
Apocynaceae	*Vinca rosea* L.
Chenopodiaceae	*Beta vulgaris* L. (Detroit dark red)
	Spinacea oleracea Mill.
Commelinaceae	*Zebrina pendula* Schnize.
Compositae	*Anthemis cotula* L.
	Aster sp.
	Calendula officinalis L.
	Chrysanthemum carinatum Schousb.
	Chrysanthemum segetum L.
	Helianthus annuus L.
	Lactuca sativa L.
	Rudbeckia hirta L.
	Zinnia elegans L.
Cruciferae	*Barbarea vulgaris* R. Br.
	Brassica arvensis (L.) Ktze.
	Brassica rapa L.
	Erysimum chieranthoides L.
	Raphanus sativa L.
	Roripa palustris (L.) Bess.
Polygonaceae	*Fagopyron esculentum* Gaertn.
Portulaceae	*Portulaca oleracea* L.
Scrophulariaceae	*Verbascum Thapsus* L.
	Verbascum blatteria L.
Solanaceae	*Browallia elata* L.
	Capsicum annuum L.
	Hyocyamus albus L.
	Hyocyamus niger L.
	Nicotiana rustica L.
	Nicotiana tabacum L.
	Petunia hybrida Viem.
	Solanum melongena L.
	Solanum tuberosum L.
Tropaeolaceae	*Tropaeolum majus* L.
Umbelliferae	*Apium graveolens* L.
Cucurbitaceae	*Citrulus vulgaris* L. (Dixie queen, Honey queen).
Gramineae	*Secale cereale* L.
	Triticum aestivum L.
	Zea mays L.
Labiatae	*Coleus blumei* Benth.
	Mentha spicata L.
Leguminosae	*Astragalus sp.*
	Baptisia sp.
	Glycine max Merr.
	Phaseolus lunatus L.
	Phaseolus vulgaris L. (Idaho refugee #5, Michelite).
	Trifolium pratense L.
	Vigna sinensis Endl.
Liliaceae	*Allium cepa* L.
Malvaceae	*Malva moschata* L.
Papaveraceae	*Eschscholzia californica* Cham.

glass slides for 72 hours, and a maximum of 298 hours was obtained in one test. The virus was no longer infective after three months in dried muskmelon leaves.

Sap from young leaves of mosaic plants remained infective in dilutions up to 1:2,500. No infection was obtained with dilutions of 1:3,000.

DISCUSSION AND CONCLUSIONS

The virus of muskmelons reported by Kendrick (4) is considered by Freitag (Middleton, 6) to be the same as the squash-mosaic virus. Presumably then, the thermal inactivation temperature of the melon strain must be the same (75° C.) as that reported by Freitag (2) for the squash-mosaic virus. The lower thermal inactivation temperature (62° C.) of the muskmelon-mosaic virus present in New York separates it from the squash-mosaic virus. The New York muskmelon-mosaic virus is distinguished from the common cucumber-mosaic virus by a more restricted suscept range and a much higher percentage of seed transmission.

The seed-transmitted melon-mosaic virus observed by Mahoney (5) in Michigan may be the same as the New York muskmelon-mosaic virus. However, in the absence of any specific data on the characters of the Michigan melon virus, and since the characters of the muskmelon virus found in New York differ from all other known viruses, the virus is described as new. The New York muskmelon-mosaic virus, as it is referred to locally, is characterized by a thermal inactivation point of the virus in expressed sap of 62° C. for a ten-minute exposure, by the veinbanding in the young leaves and the characteristic distortion and mottling in the leaves subsequently formed, and by the high percentage of seed transmission obtained in the progeny from diseased plants. Following the classification of Holmes (3) the name **Marmor melonis** sp. nov. is proposed for this virus.

The lethal virus of cantaloup occurring in the Imperial Valley of California (Middleton and Whitaker, 7) is presumably caused by a strain of cucumber-mosaic virus.

SUMMARY

A seed-borne mosaic virus of muskmelon, which has been present in New York plantings since 1936, is described as new. The disease caused by this new virus is characterized by a veinbanding and distortion of the young. leaves, and a mottling of the leaves subsequently formed, by a thermal inactivation of the virus in expressed sap of 62° C. for a 10-minute exposure, and by the high percentage of seed transmission in progeny from diseased plants. The name *Marmor melonis* sp. nov. is proposed for the New York muskmelon-mosaic virus.

DEPARTMENT OF PLANT PATHOLOGY,
   CORNELL UNIVERSITY,
      ITHACA, NEW YORK.

LITERATURE CITED

1. FIELDS, W. S. Summary of the more important plant diseases taken in connection with the insect and plant disease survey in the general vicinity of ports of entry from January, 1945 to June 30, 1945. U. S. Dept. Agr., Plant Dis. Rptr. 29: 693–699. 1945.
2. FREITAG, J. H. Insect transmission, host range and properties of squash-mosaic virus. (Abstr.) Phytopath. 31: 8. 1941.

3. HOLMES, F. O. Handbook of Phytopathogenic Viruses. 221 pp. Burgess Publishing Co., Minneapolis, Minnesota. 1939.
4. KENDRICK, J. B. Cucurbit mosaic transmitted by muskmelon seed. Phytopath. 24: 820–823. 1934.
5. MAHONEY, C. H. Seed transmission of mosaic in inbred lines of muskmelons (*Cucumis melo*). Proc. Amer. Soc. Hort. Science 32: 477–480. 1935.
6. MIDDLETON, J. T. Seed transmission of squash-mosaic virus. Phytopath. 34: 405–410. 1944.
7. ————, and T. W. WHITAKER. A lethal virus disease of cantaloup occurring in the Imperial Valley. U. S. Dept. Agr., Plant Dis. Rptr. 26: 331. 1942.
8. TAPLEY, W. T., W. D. ENZIE, and G. P. VAN ESELTINE. The vegetables of New York. Vol. 1, Part IV. The Cucurbits, pp. 1–131. J. B. Lyon Co., Albany, New York. 1937.

HOST SELECTIVITY AS A FACTOR IN THE ESTABLISHMENT OF PHYSIOLOGIC RACES OF TILLETIA CARIES AND T. FOETIDA PRODUCED BY HYBRIDIZATION[1]

C. S. HOLTON[2]

INTRODUCTION

The appearance of previously unknown physiologic races of *Tilletia caries* (D. C.) Tul. and *T. foetida* (Wallr.) Liro at more or less frequent intervals is a primary factor contributing to the perpetuation of the wheat bunt problem. This is emphasized by the fact that races frequently attract attention first by their occurrence on presumably smut-resistant varieties. Consequently it has been suggested (7) that newly introduced wheat varieties actually act as "proving ground" for races of the bunt fungi that might otherwise remain obscure. The economic implications of this theory are so obvious that the problem deserves further consideration. In a recent paper (6) dealing with pathogenicity in hybrids of the bunt fungi it was suggested that the selective influence of the host played an important part in the expression of pathogenicity and therefore in the establishment of new races. This paper presents the results of a more complete study of this problem.

REVIEW OF LITERATURE

Several workers have demonstrated the effectiveness of host selectivity in separating specific races from mixed populations of races. According to Dillon-Weston (3, 4), certain resistant varieties were rendered completely susceptible by reinoculating them with bunt spores that came from these varieties. He attributed this to the selective influence of the host on the pathogen, which operates much like a plant breeder selecting biotypes from a population of mixed breeding material. Bressman (2) obtained similar results in his studies on physiologic specialization and concluded that new virulent races of bunt could be obtained by this method. Flor (5) separated one race each of *Tilletia caries* and *T. foetida* from a mixed population by the screening influence of several winter wheat varieties. On the other hand, a race of *T. caries* that had been propagated for several generations on its own differential variety was found to be pathogenically pure, as indicated by the lack of any screening influence on its virulence. Mourashkinsky (8) showed that the passage of a given strain of *T. caries* or *T. foetida* for

five consecutive years through the same wheat variety considerably increased its virulence on that variety. He attributed this either to the screening influence of the varieties on a mixed population of races or to the production of a new race by mutation or hybridization. The latter explanation was regarded as the more probable and led Mourashkinsky (8) to the conclusion that, if such were the case, the problem of breeding wheats for bunt resistance was impossible of solution. Vielwerth (10) observed a decrease in pathogenicity of bunt populations constantly propagated on moderately susceptible wheat varieties whereas bunt populations from other varieties increased in pathogenicity when propagated on the moderately susceptible varieties. Bever (1) observed no increase in virulence on resistant varieties when pathogenically pure races were used while the pathogenicity of a hybrid population greatly increased with repeated passages through certain varieties.

For the most part these reports are concerned with results of studies in which spore populations of unknown pathogenic capabilities were used. In a study dealing with pathogenicity of hybrids between species and races of the bunt fungi (6) the writer obtained evidence that different pathogenic types were segregated and could be screened out and identified on an appropriate group of differential wheat varieties. Presentation of these results raised the question as to what selective influence, if any, would be exerted on hybrid bunt populations of this type by a completely susceptible host variety, such as Hybrid 128. Studies were undertaken to determine this point.

MATERIAL AND METHODS

Fifteen hybrids were selected for this study. This material was a portion of that used in an earlier study (6) on pathogenicity of hybrids. The wheat varieties used were Hybrid 128 (C.I. 4512), Albit (C.I. 8275), Hohenheimer (C.I. 11458), Hussar (C.I. 4843), and Hussar × Hohenheimer (C.I. 10068–1). To prevent infection by seed-borne spores from unknown sources, the seed was treated with a solution of formaldehyde (1 part to 320 parts water) for 10 minutes after which the formaldehyde was removed by washing the seed in running water.

Hybrid spores of the first generation were used to inoculate the susceptible variety Hybrid 128. Inoculum of the first segregating generation of the smut fungus (F_2) was taken from Hybrid 128 and in succeeding generations it was taken from Hybrid 128 and one or more of the other varieties. Inoculations were made by dusting the seed with spores, care being taken to prevent mixing the spores of different hybrids and different selections. The inoculated seed was planted in the field plots each fall and notes were taken the following summer. Smut percentages were based on counts of total and smutted heads to the row. The inoculations were continued for 3 years, or until the F_5 generation of the fungus was obtained, at which point the study was terminated.

EXPERIMENTAL RESULTS

It was shown in an earlier publication (6) that varietal selectivity is an important factor in the establishment of new pathogenic lines produced by hybridization. The results of this more recent study confirm that conclusion. In addition, however, these data reveal a distinct difference in the selective capacity of resistant and susceptible varieties (Table 1).

Tests were made with five hybrids between races T–8 and T–9. These races differ by the susceptibility of Albit to T–8 and its resistance to T–9 while Hohenheimer is susceptible to T–9 and resistant to T–8 (9). Hybrid 128 is susceptible and C.I. 10068–1 is resistant to both of these races. Hybrids between T–8 and T–9 were highly productive of new pathogenic entities. Some of these were more virulent and some less virulent than

TABLE 1.—*The percentages of smut produced in the F_5 generation by race hybrids of Tilletia caries, showing the selective influence of variety on the establishment of new pathogenic lines*

Smut races crossed and hybrid no.	Host variety on which inoculum was increased	Percentage smut on			
		Hybrid 128	Albit	Hohen-heimer	Sel. 10068–1
T–8 × T–9					
50a	Hybrid 128	83	46	3	1
50b	Hohenheimer	84	10	57	2
50c	Sel. 10068–1	78	21	55	14
52a	Hybrid 128	85	13	1	0
52b	Sel. 10068–1	84	67	72	34
53a	Hybrid 128	64	2	6	1
54a	Hybrid 128	91	76	0	1
54b	Sel. 10068–1	92	9	60	23
55a	Hybrid 128	84	85	4	0
55b	Hohenheimer	86	73	61	4
55c	Sel. 10068–1	87	17	84	51
T–8 × T–10					
62a	Hybrid 128	93	51	77	4
62b	Hohenheimer	86	18	81	10
62c	Sel. 10068–1	90	70	89	63

either parent, depending upon the varietal source of the inoculum. The less virulent types (Nos. 52a and 53a) were established by repeated selection of the inoculum from Hybrid 128 while Selection 10068–1 established the more virulent types (Nos. 50c, 52b, 54b, 55c). Repeated selection of the inoculum from Hybrid 128 resulted in the recovery of the T–8 parent from three hybrids (Nos. 50a, 54a, and 55a) while the T–9 parent was recovered only when the inoculum was taken from Hohenheimer (No. 50b). New pathogenic entities virulent on C.I. 10068–1 were established only by repeated selection of the inoculum from that variety (Nos. 50c, 52b, 54b, and 55c). In addition to being pathogenic on this variety, all of these except 54b were virulent on both Albit and Hohenheimer.

One hybrid between races T–8 and T–10 was studied (Table 1). These races differ from each other in the same way as T–8 and T–9 (9), except

that T–10 is more virulent than T–9 on Hohenheimer. Repeated selection of inoculum of this hybrid from the susceptible variety Hybrid 128 established a pathogenic line that combines the attributes of both parents (No. 62a) whereas a line selected from Hohenheimer (62b) was only slightly different from the T–10 parent. A new, highly virulent type was established, however, by selection from C.I. 10068–1 (62c). Thus, the results obtained with this hybrid were similar to those obtained with the hybrids between T–8 and T–9, except that no lines less virulent than either parent were obtained. Less virulent types either were not produced by this hybrid or else they were missed by the random selection of inoculum from Hybrid 128.

Thus the selective influence of the host variety is a highly significant factor in establishing new races produced by hybridization. Furthermore, the nature of this selective influence appears to depend largely upon the degree of resistance of the varieties. Apparently the highly susceptible varieties, such as Hybrid 128, tend to promote the increase and establishment of segregates with low virulence. On the other hand, the highly resistant varieties, such as C.I. 10068–1, tend to promote the establishment of the more virulent types. This seems logical in view of the fact that only the highly virulent lines can infect the resistant varieties. However, there appears to be no reason why the highly susceptible variety should tend to select the less widely virulent types, unless these are produced in greater abundance than the more virulent types.

These results are substantiated in part by practical experience in the introduction of new bunt-resistant wheat varieties. As pointed out in an earlier paper (7) the establishment of a new, smut-resistant wheat variety usually is followed, sooner or later, by the appearance of a bunt race capable of infecting that variety. Consequently, in areas where new varieties are introduced, often the population of races is greater than in areas where long-established, highly susceptible varieties are grown (9). In other words, the results of this study, together with practical experience, emphasize again the need for a continued program of breeding resistant varieties and the development of more efficient seed-treatment practices if bunt of wheat is to be effectively controlled.

CONCLUSIONS

1. Evidence is presented which shows that the selective influence of the host is an important factor in the establishment of physiologic races of the bunt fungi produced by hybridization.

2. The highly susceptible variety Hybrid 128 tends to promote the establishment of races with low virulence.

3. Highly resistant varieties tend to promote the establishment of races with high virulence.

4. These results are substantiated by practical experience. The population of highly virulent races is greatest in regions in which many new smut-resistant varieties are grown.

5. This study reemphasizes the importance of a continuous program of investigation on the bunt problem, embracing the genetics of bunt resistance, breeding bunt-resistant varieties, the biology of the bunt fungi, and seed treatment method and practices.

U. S. DEPARTMENT OF AGRICULTURE,
AGRICULTURAL EXPERIMENT STATION,
PULLMAN, WASHINGTON.

LITERATURE CITED

1. BEVER, W. M. Reinoculation of resistant varieties of wheat with purified physiologic races of *Tilletia tritici* and *T. levis*. Phytopath. 29: 863–871. 1939.
2. BRESSMAN, E. N. Varietal resistance, physiologic specialization, and inheritance studies in bunt of wheat. Ore. Agr. Exp. Sta. Bul. 281. 1931.
3. DILLON-WESTON, W. A. R. Resistance of wheat varieties to bunt (*Tilletia tritici*). Nature 123: 243. 1929.
4. ————. The relative resistance of some wheat varieties to *Tilletia tritici*. Ann. Appl. Biol. 19: 35–54. 1932.
5. FLOR, H. H. Studies on physiologic specialization in *Tilletia tritici* and *T. levis* in the Pacific Northwest. Jour. Agr. Res. [U.S.] 47: 193–213. 1933.
6. HOLTON, C. S. Extent of pathogenicity of hybrids of *Tilletia tritici* and *T. levis*. Jour. Agr. Res. [U.S.] 65: 555–563. 1942.
7. ————, and F. D. HEALD. Studies on the control and other aspects of bunt of wheat. Wash. Agr. Exp. Sta. Bul. 339. 1936.
8. MOURASHKINSKY, K. E. [Effect of source of origin of *Tilletia tritici* and *T. levis* spores on the susceptibility of wheat to infection with bunt. II.] [Diseases of cereal crops.] Siberian Scient. Res. Inst. Cereal Crops. 1932. Abstr. in Rev. Appl. Mycol. 12: 153–154. 1933.
9. RODENHISER, H. A., and C. S. HOLTON. Physiologic races of *Tilletia tritici* and *T. levis*. Jour. Agr. Res. [U.S.] 55: 483–496. 1937.
10. VIELWERTH, V. [The development of physiologic forms of the smooth-spored bunt (*Tilletia levis*) on moderately susceptible wheats.] Ochr. Rost. 14(55): 66–70. 1938. Abstr. in Rev. Appl. Mycol. 17: 511. 1938.

VIRUS ATTENUATION AND THE SEPARATION OF STRAINS BY SPECIFIC HOSTS[1]

JAMES JOHNSON

Some of the most obscure and important problems relating to viruses are associated with the origin and utilization of attenuated or mild strains of specific viruses. The protective or immunizing action of mild strains against more severe strains of the same virus is well known among virus diseases of both animals and plants. The causal agent of smallpox, for example, was known to be subject to attenuation by passage through cows or calves long before the virus character of disease was known. The true nature of such attenuation, transformation, modification, or alteration of a virus in either plant or animal hosts appears not to have been conclusively demonstrated. Similarly, little is definitely known about the origin of virus strains having greater virulence than the parent virus. In recent years there has been repeated evidence of the existence of mixtures of many virus strains in any ordinary virus culture. It has been generally accepted also that these strains, including attenuated strains, may arise through natural or induced mutation of the respective parent viruses. Such parent virus mixtures have been subdivided into other related group mixtures on the basis of symptomatology. The possibilities of isolating pure strains of viruses, originating for all practical purposes from single virus particles, offer new opportunities for securing more information on attenuation and mutation. The nature of the transformation of specific viruses from one degree of virulence to another on passage through suitable hosts is especially in need of investigation in order to promote more agreement in terminology (1, 17). The results with the ordinary tobacco-mosaic virus show that attenuation is due to the selective or differential response of a specific host to a mixture of severe and mild strains of this virus, permitting the separation of the latter from the former. According to the present results, the changes or mutations that created the mixture of strains occurred prior to the separation or attenuation by the host plant.

An abstract of this paper has been published (10).

SINGLE-LESION PURE-LINE STRAINS

Single-lesion strains often have been used in tobacco-mosaic-virus studies but little continued effort has been made to secure pure lines. During the course of the studies on attenuation, it became desirable to isolate and maintain single-lesion pure-line strains of tobacco-mosaic virus differing in their capacity to produce severe symptoms of disease. Until comparatively recently, a pure strain of a virus referred to a culture free from any other

distinct or unrelated virus. Ordinary virus mixtures were easily separated by the use of differential hosts, properties, or vectors. However, it long has been known that different, though related, strains of a single virus frequently coexisted in ordinary virus cultures. Some workers believe that viruses are continually mutating and, on this basis, it would hardly be possible to maintain a culture that is not a mixture of strains (7, 15). This assumption is easily subject to experimental tests with the tobacco-mosaic virus and, in our experience, mutation is far less frequent than is generally supposed.

The single lesions produced on the hybrid host (*Nicotiana tabacum* × *N. glutinosa*), at virus dilutions as high as 1 to 10,000,000, are ideal for initiating pure-line cultures. The method is comparable to the Petri-dish culture method for isolating strains of bacteria and fungi. Hence, problems similar to those existing in Petri-dish isolations arise with virus isolation, and some improvements in methods need to be made and additional precautions taken in order to secure reliable virus isolations. The local-lesion host inoculated, for example, should be highly susceptible to infection and the virus applied at high dilutions together with frequent consecutive transfers from single lesions. The tobacco hybrid host is the most suitable yet found for this purpose. It is preferable to start with an aged, diluted, and buffered inoculum to lower the clumping of the virus particles. Gentle wiping with a cheesecloth pad on a carborundum-treated leaf, followed by rinsing with water, permits a possible selection of single-particle local lesions in the first transfer. The single lesions, removed with a small cork borer (5/16 in.), are thoroughly macerated and highly diluted (from 1 : 10,000 to 1 : 100,000) for transfers. Several consecutive transfers to the hybrid and repeated checking on the ordinary tobacco host is essential to determination. Proof of a pure strain cannot be finally conclusive in the absence of actual physical single-particle manipulation; hence, reasonably satisfactory proof must depend upon continued transfer through different host plants without evidence of further separation into other strains except such as may be attributed to natural or induced mutation.

Extensive trials with single-lesion tobacco-mosaic strains have resulted in a remarkable constancy in symptom expressions in the individual strains. Interesting though rare exceptions were noted, such as the occurrence of a trace of yellow-mosaic strain in a pure-line strain. Attempted pure-line isolation of this yellow-mosaic strain by the single-lesion method could not be accomplished, although the strain could be increased in relative concentration. It may be assumed that the occurrence of the yellow strain resulted from contaminating particles inseparable from other virus particles present in only extreme dilution in the original isolations. The conclusions of McKinney (15) and others as well as our own observations on the yellow strain suggest that it is more likely to be a recurring mutation.

The strains apparently occurring with greatest frequency in an ordinary field or laboratory virus mixture of ordinary tobacco-mosaic virus are

those which may be classified as the "severe" and "mild" strains, with several intermediary strains (Fig. 1). Our severe strain corresponds with strains described by others under the same name or other names indicating malformation, such as "distorting" strain. As yet it is not possible, on

FIG. 1. Severe (A), medium (B), and mild (C) strains of virus on tobacco plants showing typical symptoms from single-lesion isolations. Photograph by Eugene H. Herrling.

FIG. 2. The sea holly (*Eryngium*) host plant used in investigations on attenuation. A. Virus-free plant. B. Plant inoculated with tobacco-mosaic virus. No definite leaf symptoms result from this virus, except stunting, under some conditions, as shown here. Photograph by Eugene H. Herrling.

the basis of symptom expression alone, to state that one strain is completely identical with another arising from a different isolation; yet, unless one admits of no constancy in the nature of viruses, single-lesion pure-line strains may be grouped into a relatively small number of strains as far as reliable determination is concerned. Having obtained single-lesion pure-line strains of severe and attenuated viruses, it was possible to use and compare them on alternate hosts or to subject them to other treatments aimed at demonstrating attenuation or mutation. ·

THE NATURE OF ATTENUATION IN ERYNGIUM

In connection with efforts to unravel a puzzling mixture of viruses in the perennial sea holly or Buttonsnakeroot (*Eryngium aquaticum* L.), it was found that one of the viruses frequently present was that of ordinary tobacco mosaic (Fig. 2). The determination of the disease complex in sea holly was made more difficult by the fact that invariably the tobacco-mosaic virus component was present as a mild or attenuated strain. Laboratory inoculations consequently were made to sea holly with ordinary tobacco-mosaic virus, yielding severe symptoms. Infection occurred on the inoculated leaves and the original type of virus was recoverable from the inoculated symptomless leaves. The new, noninoculated and symptomless leaves of this slow-growing plant were repeatedly tested and eventually yielded virus. The virus recovered from these leaves was clearly not the same virus type as had been used for inoculation. Instead of yielding the severe symptoms of the parent virus on tobacco, the symptoms were always characteristic of a mild type. The virus originally yielding the severe disease on tobacco had been modified, transformed, or altered by the sea-holly host. If the parent virus is assumed to have mutated, all of the virus originally introduced must have undergone change, a supposition quite out of line with the accepted meaning of mutation. Consequently, some more logical explanation was sought for this behavior. One obvious method of investigation was to inoculate virus-free sea-holly plants separately with single-lesion pure-line strains of severe and attenuated tobacco-mosaic virus. After first testing the plants to prove freedom from virus, one series was inoculated on lower marked leaves with only a mild virus strain, and a corresponding series with a severe virus strain. At about weekly intervals, the inoculated leaves and the new leaves were tested for virus by removing small leaf discs with a cork borer, crushing this tissue in a small amount of water (1 cc. per disc), and inoculating to the tobacco-hybrid test plant, yielding local lesions. In this manner the progress of infection could be followed in the host. Reinoculations from local lesions back to ordinary tobacco determined whether the mild or severe strain of the virus had been recovered. In many cases the results were simplified by the fact that the mild strain yielded definitely smaller lesions than did the severe strain on the same leaf of the hybrid (Fig. 3). The results of a series of tests are shown in table 1. The severe form multiplied slowly but did not move out

of the inoculated leaves into new leaves. Neither did the severe strain from this source yield any transformed single-lesion isolations. The mild form multiplied more rapidly in the inoculated leaves, moved into the new leaves, and remained as mild virus during the full year of these reisolations (Table 2). Some minor exceptions of undetermined significance
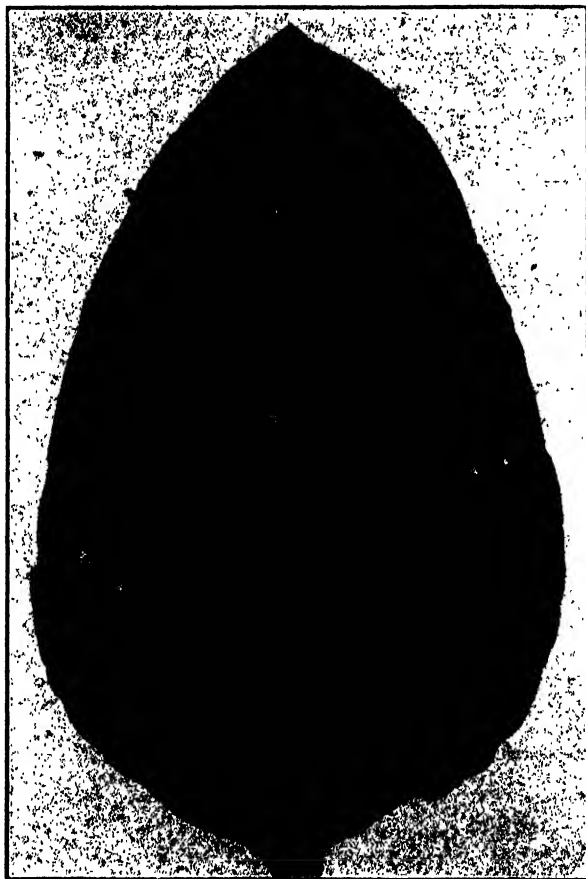
Fig. 3. A leaf of the tobacco hybrid host (*Nicotiana tabacum* x *N. glutinosa*). The left half of the leaf was wiped with a high dilution of the severe strain of tobacco mosaic and the right half with a mild (attenuated) strain. Note the smaller lesions on the right half (upper half in the illustration). Photograph by Eugene H. Herrling.

occurred during the later period of the tests. The sea-holly host is thus capable of separating (screening or filtering) two closely related strains of the same virus inoculated simultaneously to the plants. This separation is comparable to the longer known and more easily demonstrated separation of distinct and unrelated viruses by specific hosts.

Since, in the preliminary tests, an ordinary strain mixture of the tobacco-mosaic virus was inoculated to the sea holly and only a mild strain was recovered, the original virus was clearly attenuated according to the

accepted definition of this term since the work of Jenner (6) and Pasteur and his colleagues (16). The attenuation in *Eryngium* is thus shown to be only a matter of separation due to failure of the severe form of the virus to become systemic and to move into the new or noninoculated tissue. That other plants are capable of yielding similar responses with the tobacco-mosaic virus is probable, but the limited search for such plants has not been fruitful. Belladonna (*Atropa belladonna* L.), potato (*Solanum tuberosum* L.), digitalis (*Digitalis lanàta* Ehrh.), and tomato (*Lycopersicon esculentum* L.) were tested in some detail with negative results in this direction. Single-lesion isolations from inoculated infected leaves or from noninoculated leaves, if infected, yielded no attenuated virus, as illustrated by belladonna (Table 3).

TABLE 1.—*Summarized results of tests for virus in inoculated and noninoculated leaves of Eryngium made at intervals over a period of 7 months after inoculation of marked leaves with single-lesion pure-line severe and attenuated strains of the ordinary tobacco-mosaic virus*

Virus strain inoculum	Plant designation	Inoculated (lower) leaves			Noninoculated (new) leaves		
		Number of tests	Number yielding virus	Strain of virus recovered	Number of tests	Number yielding virus	Strain of virus recovered
Severe	A	4	2	Severe	11	0	....
	B	4	3	Severe	5	0	....
	C	3	2	Severe	7	0	....
	D	3	1	Severe	6	0	....
	Total A, B, C, D	14	8	Severe	29	0	....
Attenuated (mild)	F	5	5	Mild	6	6	Mild
	G	3	3	Mild	5	5	Mild
	H	2	2	Mild	4	4	Mild
	I	2	2	Mild	5	5	Mild
	Total F, G, H, I	12	12	Mild	20	20	Mild

Further investigations on the sea holly have revealed that the severe strain of the virus in the inoculated and infected leaves eventually may become lower in concentration or disappear from these leaves. The older leaves, in any case, finally will die and be cast off the plant. Thus the host actually recovers from infections with the severe strain of the virus; and plants, once infected with the severe strain only, again may become susceptible to new infection with the same or other strains. The attenuated strain, on the other hand, persists in the host and protects it against the severe strain. The attenuated strain not only infects all the above-ground tissues, which are normally destroyed by freezing to the ground level during the winter, but permeates the crown and roots of the perennial sea holly and develops in the new spring growth. Some difficulty has been experienced in obtaining infection on some individual plants of *Eryngium*, indicating high resistance to both strains of the virus. Upon reinoculation (Table 2,

TABLE 2.—*Summarized results from reinoculations of new leaves of the same Eryngium plants as shown in table 1 with single-lesion pure-line strains of tobacco-mosaic virus. Tests for virus made at intervals 7 to 12 months after first inoculation*

Plant	Inoculation and strain of virus			Inoculated leaves			Noninoculated leaves		
	First April 11, 1946	Second Oct. 28, 1946	Third Dec. 7, 1946	Number of tests	Average number of lesions on 5 half-leaves of hybrid	Strain of virus recovered	Number of tests	Average number of lesions on 5 half-leaves of hybrid	Strain of virus recovered
A, B, C	Severe	Severe	None	20	340	Severe	18	3	Severe
D, E	Severe	Severe	Mild	9	453	Severe after first and second inoculation; mild after third	11	869	Mild
F, G, H, I	Mild	Severe	None	8	1222	Mild after first; severe after second	5	710	Mild
J	Mild	Severe	Mild	3	166	None after first and third; undetermined after second[a]	11	2	Mild

[a] A significant amount of infection was found only on Dec. 6; plant J was resistant to systemic invasion by both strains.

plants D, E, J) only one plant (J) proved resistant to systemic infection with the mild strain.

SEPARATION OF A SEVERE STRAIN ON TOMATO

The sea-holly host accounts for the natural separation and occurrence of mild strains of a virus as distinct from the more common and severe mix-

TABLE 3.—*Summarized results of tests for virus in young tomato and belladonna plants at intervals of 2 to 22 days following inoculation of lower marked leaves with severe and attenuated strains of the ordinary tobacco-mosaic virus*

Test plant and strain of virus inoculum	Number of plants inoculated	Inoculated leaves				Noninoculated leaves		
		Number of tests for virus	Average number of lesions on 5 half-leaves of hybrid	Strain of virus recovered		Number of tests for virus	Average number of lesions on 5 half-leaves of hybrid	Strain of virus recovered
Tomato								
Severe	5	5	840	Severe		7	1400	Severe
Attenuated (mild)	16	23	1.5	Mild		13	0.8	Mild
Belladonna								
Severe	8	8	786	Severe		30	1	Severe
Attenuated (mild)	8	8	370	Mild		24	3	Mild

tures. The reverse situation also has been suspected in the field and laboratory. Occasionally, severe strains of specific virus diseases (evidently obtaining no protective influence from mild strains infecting simultaneously) occur naturally, almost as though single-lesion laboratory selection had preceded. Such a selection by a host plant has been found to occur with the ordinary tobacco-mosaic virus on the tomato. When lower marked leaves of tomato (*Lycopersicon esculentum* L. var. Marglobe) are inoculated with virus of the ordinary severe tobacco mosaic, the severe mosaic virus is recoverable from both the inoculated and noninoculated leaves. When a single-lesion pure-line severe strain of the virus was used, the results were the same; however, when a single-lesion pure-line mild strain of the tobacco-mosaic virus was used for inoculum, the results were strikingly different. The virus could not be recovered from either the noninoculated or the inoculated leaves (Table 3). The situation was the reverse of that in *Eryngium* since the severe strain became systemic and differed also in the fact that the mild (attenuated) strain of the virus did not infect, the tomato plants used being naturally immune. The separation of the two strains, therefore, occurred at the point of inoculation rather than in the tissue itself. It cannot be said as yet that all tomato varieties are immune from any one attenuated strain or that one tomato variety is immune from all attenuated strains, although this seems likely. This behavior on tomato is highly indicative of the manner in which virus strains of high virulence may arise under natural conditions as contrasted to methods of laboratory isolation.

A limited number of other plants have been investigated for reactions similar to that of the tomato but none has been found. The considerable number of known solanaceous hosts (4, 5), as well as those outside this family, suggests other possibilities. Many of these are susceptible, either locally or systemically, without the development of symptoms. The testing of many species with different strains of viruses, when involving single-lesion reisolation to determine possible modifications of the virus itself including that in symptomless tissues, is a laborious and time consuming task. However, there are possibilities of developing new and short-cut methods, as is illustrated by the discovery of more reliable differentiation of the local lesions themselves, than is possible on the hybrid host normally used for that purpose in our tests.

SELECTIVE LOCAL-LESION REACTIONS BY ATTENUATED STRAINS

Wiping the leaves of the tobacco variety Havana 38 with either the attenuated or severe strains of tobacco-mosaic virus yields no inoculative local necrotic lesions or symptoms but only the typical systemic symptoms of mottling, stunting, or malformation. This type of response is characteristic of most of the common commercial varieties of *Nicotiana tabacum*. The hybrid *N. glutinosa* × *N. tabacum*, regularly used as the local-lesion test plant in our laboratory, yields only local lesions on the older and larger

plants used for this purpose. If young hybrid plants are used, however, the severe strain produces systemic necrosis, entering the veins, midrib, stem, and bud, and often killing the plant to the soil level. The attenuated strains, however, remain localized even in young plants, indicating less capacity for movement. When the age and condition of the hybrid host are favorable, both strains remain definitely localized, but the lesions of the severe strains may be larger than those of the attenuated strains, making it relatively simple to select and isolate strains of different degrees of severity from a mixed virus culture. When the single-lesion pure-line severe and attenuated strains are wiped on opposite sides of the same hybrid leaf,

FIG. 4. The left side of each of the three leaves shown was inoculated with a severe tobacco virus strain and the right side with a mild (attenuated) strain of the same virus. A. The *tabacum-glutinosa* hybrid. B. Var. Daruma. C. A *tabacum-longiflora* hybrid. Note the different reactions for the severe strain of the virus on the left sides of the leaves. Photograph by Eugene H. Herrling.

the uniformity of the difference in size of the lesions is often striking (Fig. 3). Selection on the basis of size of lesion, however, is not a reliable criterion.

During a series of tests by the half-leaf wiping method, similar inoculations were made on a foreign variety of tobacco known as Daruma. Strangely enough, good necrotic local lesions developed on Daruma with the mild or attenuated strain but no local lesions developed from the severe strain on the opposite sides of the same leaves (Fig. 4). The severe strain infects Daruma systemically or produces only faint chlorotic areas on the inoculated leaf. The mild strain is also capable of systemic infection on young plants of Daruma. This unexpected response naturally suggested

similar tests on other varieties of *Nicotiana tabacum* and other species of *Nicotiana*. It was found that some species of *Nicotiana* responded in a similar manner to Daruma, *i.e.*, yielding necrotic lesions with only the mild strain. These species included chiefly *N. sylvestris* and *N. longiflora*. Other species of *Nicotiana* yielded local lesions with both strains (*N. glutinosa, N. rustica*) and others with neither strain, as is the case with most varieties of *N. tabacum*. Some other varieties of *N. tabacum*, like Daruma, yielded large or incipient local lesions with the mild strain (*e.g.*, Maryland Broadleaf, Xanthia, and Orinoco). The F_2 of a cross between *N. longiflora* and *N. tabacum* (Clayton's T.I. 106) yielded good local lesions with the mild strain only (Fig. 4). The parentage of Daruma is not known to us, but its appearance and virus response suggest that it possesses both *N. tabacum* genes and genes for local lesions with the mild strain of the tobacco-mosaic virus which originally was most likely derived from some other species than *N. tabacum*.

VIRUS MUTATIONS INDUCED BY HEAT.

The yellow tobacco-mosaic virus apparently arises in nature as a mutant strain in the absence of any known contributing factor, unless it is more likely to develop on some hosts than on others. In a series of consecutive transfers of a single-lesion pure-line severe strain, through the hybrid and tobacco, one set of inoculations included 2 Havana and 1 Burley plant. A distinct yellow variant appeared on the Burley but not on the Havana plants, suggesting that the low-chlorophyll Burley had contributed to its origin, but no further evidence on this point was secured. We have observed, however, that some varieties of tobacco yield highly intensive symptoms of yellow mosaic as compared to others. McKinney (15) and Jensen (7) have concluded that the yellow mosaic forms arise occasionally as mutants rather than as contaminants. Earlier attempts made in this laboratory (11) to modify viruses themselves by passage through different hosts yielded only negative results except with Martinya, but single-lesion methods were not used at that time. The reported attenuation of the sugar-beet curly-top virus (2) in weed hosts has been questioned and apparently disproved (3, 14). As indicated above, we have sought for virus modification of pure-line severe strains by certain uncongenial hosts with only negative results. There appears to be no conclusive instance of mutation occurring in host tissue in which the virus multiplies except that reported for yellow tobacco mosaic virus.

The number and frequency of attenuated strains in field samples of tobacco mosaic suggest that some contributing conditions favor their occurrence. We have obtained mild strains from virus-infested soil and from aerated and fermenting extracts, indicating that extensive modification of virus particles may be concerned (9). In these earlier trials, single-lesion pure-line strains were not used, and it is quite as likely that some form of separation of severe and mild forms was occurring as that new strains were

formed. Attenuated strains (8) were obtained through the action of high temperatures (35°–37° C.) on virus infected plants but, again, the original inoculum was not pure-lined or known to be a strain free from mild strains.

Conclusive proof that a pure-line virus strain could be modified in any manner, therefore, remained to be secured. In view of our earlier trials, it seemed likely that this could be accomplished by allowing a single-lesion pure-line strain of severe tobacco virus to multiply at a constant temperature of about 36° C. Lower marked leaves or portions of leaves of several tobacco plants, consequently, were inoculated with a severe strain and incubated at 35°–37° C. in an artificially lighted air-control chamber for plant culture. Sample discs of both inoculated and noninoculated leaves were taken at intervals with a small cork borer and the discs either stored for future use on filter paper in covered Petri dishes or inoculated at once at a suitable dilution to the hybrid host which yielded local lesions. These local lesions were then selected according to size or appearance and inoculated to Havana 38 tobacco in comparison with the same strains that had not been incubated to determine the modifications, if any, that had resulted from the heat exposures.

The results showed conclusively that distinct modification developed in some particles of the pure-line virus during the incubation at 35°–37° C. The most frequent variants were in the direction of strains of reduced virulence (attenuation), although this may have been due in part to more frequent selection of small lesions for transfer (Table 4). Many of the

TABLE 4.—*The type and number of single-lesion strains isolated from Havana tobacco plants grown at 35°–37° C. after being inoculated with a single-lesion pure-line severe strain of tobacco mosaic. The periods of temperature treatment varied from 5 to 13 days, and the lesions isolated on the hybrid host were usually selected for relative size. Recovered strains were determined on the Havana variety*

Exp.	Number of plants at 35°–37° C.	Number of plants yielding attenuated virus	Number of small local lesions yielding no infection	Strains and number of times isolated from single lesions			
				Very mild	Mild	Severe	Undescribed or new
1	6	6	5	6	18	16	1
2	8	6	6	8	6	23	5

local lesions were very small and some, even when combined to yield more inoculum, failed to yield any infection whatever on transfer to tobacco. This suggested the development of strains capable of causing weak infections on the hybrid but too weak to be transferred further. Other lesions yielded no symptoms on tobacco but systemic infection was present; that is, the virus was latent in tobacco as could be demonstrated by inoculation back to the hybrid host where it yielded abundant local lesions. Other lesions yielded varying degrees of attenuation which could be perpetuated as

single-lesion pure-line strains in successive transfers. The majority of the lesions isolated from noninoculated leaves in the temperature-control chamber were evidently of the original severe strain, as judged by the size of the local lesion obtained, only about 5 per cent showing evidence of being transformed to the mild strain. The results in 2 series of temperature exposures were not the same in all plants tested, modification being greater in some plants than in others. Since only relatively small areas of treated plants could be sampled, and the sample discs were purposely selected from different leaf-positions and parts of leaves, much variation in this respect was to be expected. More detailed work would need to be done to trace the rate and type of changes developing.

The most surprising result of the heat treatment of the virus was the appearance of entirely new strains, not easily recognized as tobacco-mosaic
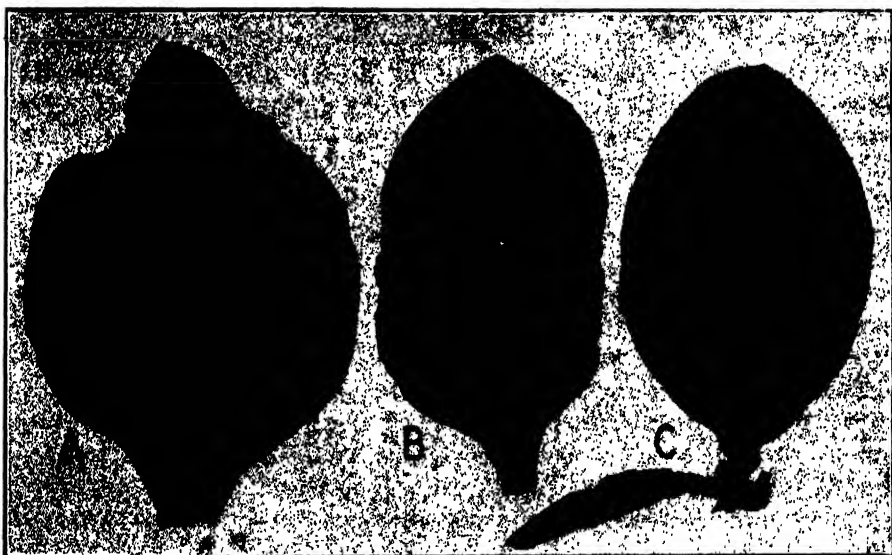
FIG. 5. Tobacco leaves infected by three single-lesion strains (A, B, C) of modified or mutated virus, originating from a single-lesion pure-line strain of severe tobacco-mosaic virus when grown for several days at 35°–37° C. These new strains, showing little or no resemblance to the parent virus, remained constant in continued successive transfers. Note especially the bud-infection symptom on C. Photograph by Eugene H. Herrling.

virus, some being more severe in certain respects on ordinary tobacco than the original severe malforming strain used for inoculum. Proof of their identity as single-lesion strains of the tobacco-mosaic virus was verified by the production of ordinary appearing local lesions of tobacco virus on the hybrid and *Nicotiana glutinosa* hosts and by thermal death-point and virus-aging determinations. One of these strains was referred to as the "bud-infecting" strain for the reason that only the bud of young plants became very chlorotic and usually was killed (Fig. 5). New, secondary buds were then formed, developing scraggly plants with malformed leaves but with little further evidence of continued necrosis or chlorosis on the older plants.

Other new strains were milder and the virus less concentrated, but they seemed to have some similarities to the bud-infecting strain (Fig. 5).

With the heat treatment, therefore, the origin by mutation of new strains of a virus from a single-lesion pure-line strain is believed to be demonstrated. No doubt similar mutations might be procured from single-lesion pure-lines by other methods as, for example, by chemical and X-ray or ultraviolet ray treatments. When single-lesion pure-line strains are not used, proof would need to be established that the transformation is not due to some form of separation of mixed strains as a consequence of differential sensitivity to the treatment. On the basis of our experience with single-lesion pure-line strains, there is little reason to doubt that some of these may be said to have come from a single virus particle.

DISCUSSION

Perhaps the greatest interest attached to the present investigations relates to the nature of attenuation in the broadly accepted sense of the term. Attenuation normally refers to the modification or transformation of a virus yielding severe types of symptoms or disease on its normal host to one yielding only mild symptoms on the same host. The attenuated virus, when inoculated to the host prior to or at the time of infection with the severe strain, normally protects the host from severe attacks of the severe strain. Among animal viruses, this principle has found wide application. Although attenuation has been obtained in other ways, the first and most common method is by passage of the virus to be attenuated through an alternate host or hosts that will yield the desired result. The virus must obviously be modified 100 per cent; otherwise the use of protective strains might be ineffective or even dangerous with certain diseases. It has been assumed, in some cases at least, that the alternate host causes the virus to "mutate," a term defined as a sudden change from the normal which is capable of continued perpetuation. A form of mutation, which is complete for the entire population of individual organisms or virus particles concerned, has not been demonstrated and is most unlikely. The use of such terms as "modification," "transformation," "alteration," or even "attenuation" has not implied anything specific as to the origin of mild strains of viruses. Since the early work on smallpox by Jenner (6) and on rabies by Pasteur (16), the nature of attenuation seems to have remained in some obscurity. There is ample proof of the existence of attenuated virus strains in plants, and their immunizing action against related, more severe strains of virus is closely comparable to the behavior of the animal virus strains. Since the term "attenuation" has been adopted for plant viruses from its usage in the animal virus field, additional reasons exist for comparing and correlating results in the two branches of research.

Attenuation of the ordinary tobacco-mosaic virus as it exists in nature by *Eryngium* (sea holly) is a result of the separation, screening, or filtering out of mild or attenuated strains from the more severe strains. Only the

mild strain is normally recoverable from the noninoculated tissues of the plant. The mutation of virus particles from which the attenuated strain is derived, must have occurred previously, so that the two strains existed as a mixture some time prior to infection and passage through the attenuating host.

Further support of this conclusion is to be found in evidence presented that related virus strains in mixture may be separated on the tomato plant on the basis of selective susceptibility to infection. In this instance the separation is reversed, the mild strains are eliminated, the severe strains are isolated and their occurrence in nature among milder strains thus is explained. Such natural occurrences of mild and virulent strains in a plant population are very suggestive of a similar situation in animal viruses.

The reversal of the behavior of related virus strains on genetically related hosts indicates a variety of new problems in need of further study, especially with respect to the inheritance of resistance in different strains of plants to different strains of the same virus. The production of new strains of viruses from pure-line strains by the simple process of the heat treatment of the multiplying virus also opens new avenues for basic research on mutation in viruses.

The separation of the virus strains on sea holly and on tomato are sharp and complete reactions. Separation of virus strains may be accomplished occasionally by hosts in which the rate of progress of infection into new tissues differs less strikingly. Such an instance was essentially accomplished in this laboratory by Koch (12) in dealing with the potato mottle and the potato ring-spot strains. In this and similar cases the purity of the isolations is less distinct and certain, even after repeated passage through the same host, and the alteration may be only one of relative concentration. Many viruses have been changed in virulence or in symptom expression by selections of this sort, but complete freedom of one strain from another is not assured unless the host is naturally highly selective or may be made more selective under special conditions such as a constant change in environment. In view of the local-lesion reaction of attenuated strains on *Nicotiana sylvestris* in our experiments, it would be interesting to repeat some of Kunkel's (13) experiments on acquired immunity in which he used *Nicotiana sylvestris* as a host plant.

SUMMARY

The true nature of attenuation as applied to plant and animal viruses has not been conclusively determined. In a similar manner, the natural origin of apparently new or highly virulent strains of viruses from normal parent-viruses has remained obscure.

The well known ordinary tobacco-mosaic virus offers exceptional possibilities for investigating such phenomena. Through the use of proper methods and precautions, single-lesion pure-line strains may be isolated that, for all practical purposes, arise from single virus particles. The

pure-line strains remain remarkably constant, although it cannot be said that occasionally alterations do not occur as a consequence of mutation rather than as a result of contamination. Pure-line strains are essential to reliable studies on the nature of attenuation and mutation in viruses.

Naturally infected sea-holly (*Eryngium*) plants regularly yielded an attenuated strain of the tobacco-mosaic virus. Laboratory plants inoculated with an ordinary field culture of severe tobacco-mosaic virus yielded only an attenuated strain in the noninoculated or new leaves, although the severe virus infected the inoculated leaves as well. *Eryngium* plants inoculated with pure-line strains of severe and attenuated virus separately showed that the severe strain remained localized in the inoculated leaves and the attenuated strain became systemic. Consequently, the attenuation of the ordinary tobacco-mosaic virus is due to the ability of *Eryngium* to separate an attenuated strain from a severe strain when the two strains exist in combination.

In a similar manner it has been shown that the tomato is capable of performing the reverse separation; namely, of isolating a severe strain of the tobacco-mosaic virus from an attenuated strain of the same virus present in a mixture. Such selective capacity demonstrates a means by which more virulent strains may arise under natural conditions as contrasted to laboratory isolation. The loss of the immunizing influence of the attenuated strains also adds to the virulence of the separated severe strain.

The attenuated strains yielded good local-necrotic lesions on certain species and varieties of *Nicotiana*, whereas the severe strain did not produce lesions on these same hosts. This behavior offers an exceptionally good method for determining the presence of either the attenuated strain or the severe strain or both in any sample extract, especially when combined with inoculation to the ordinary hybrid host.

It has been shown that single-lesion pure-line strains of the tobacco-mosaic virus, normally remaining constant, may be induced to mutate at a relatively high rate by allowing virus multiplication to occur at temperatures of 35°–37° C. Both attenuated and new strains have been isolated in this manner.

UNIVERSITY OF WISCONSIN,
 MADISON, WISCONSIN.

LITERATURE CITED

1. BURNET, F. M. Immunization against epidemic influenza with living attenuated virus. Med. Jour. Aust. 3: 385–399. 1943.
2. CARSNER, E. Attenuation of virus of sugar beet curly top. Phytopath. 15: 745–757. 1925.
3. GIDDINGS, N. J. Curly top virus strains. (Abstr.) Phytopath. 30: 786. 1940.
4. GRANT, T. J. The host range and behavior of the ordinary tobacco mosaic virus. Phytopath. 24: 311–336. 1934.
5. HOLMES, F. O. A comparison of the experimental host ranges of the tobacco-etch and tobacco-mosaic viruses. Phytopath. 36: 642–659. 1946.
6. JENNER, EDWARD. An inquiry into causes and effects of the variolae vaccinae, a disease discovered in some of the western counties of England, particularly Gloucestershire and known by the name of the cow pox. London. 1798.

7. JENSEN, J. H. Studies on representative strains of tobacco-mosaic virus. Phyto-path. 27: 69–84. 1937.
8. JOHNSON, J. The attenuation of plant viruses and the inactivating influence of oxygen. Science 64: 210. 1926.
9. ——————. Further studies on the attenuation of plant viruses. (Abstr.) Phytopath. 18: 30. 1928.
10. ——————. Virus attenuation and mutation. (Abstr.) Phytopath. 37: 12. 1947.
11. ——————, and T. J. GRANT. The properties of plant viruses from different host species. Phytopath. 22: 741–747. 1932.
12. KOCH, K. L. The nature of potato rugose mosaic. Phytopath. 23: 319–342. 1933.
13. KUNKEL, L. O. Studies on acquired immunity with tobacco and aucuba mosaics. Phytopath. 24: 437–466. 1934.
14. LACKEY, C. F. Restoration of virulence of attenuated curly top virus by passage through susceptible plants. Jour. Agr. Res. 55: 453–460. 1937.
15. MCKINNEY, H. H. Evidence of virus mutation in the common mosaic of tobacco. Jour. Agr. Res. 51: 951–981. 1936.
16. PASTEUR, L., CHAMBERLAND, and ROUX. Sur une maladie nouvelle provoquée par la salive d'un enfant mort de la rage. Compt. Rend. Acad. Sci. 92: 159. 1881.
17. RIVERS, T. M. Filterable viruses. The Williams and Wilkins Co., Baltimore. 428 pp. 1928.

THE RELATION OF MEADOW NEMATODES TO BROWN ROOT-ROT OF TOBACCO[1]

W. D. VALLEAU AND E. M. JOHNSON

For many years a root-rot of tobacco that differs from black root-rot has been under observation in Kentucky. The dead roots are brown, or sometimes pink or purple, instead of black, and the cortex is usually reduced to a thin sheath loosely surrounding the stele. Growth of tobacco is very slow following transplanting. The leaves may wilt in the heat of the day. Roots of the original root system usually die early. Later, toward the middle of summer, new roots develop on the buried stems and plant growth is fairly normal if the soil is fertile. Some years there is a marked difference in wilting of tobacco following grass and following tobacco, the latter remaining turgid on hot days while tobacco following orchard grass, for instance, wilts severely. Other years there seems to be little, if any, difference. That the trouble is not caused alone by turning under sod is indicated by the fact that tobacco following an old bluegrass sod, on the Station farm, that probably never before had been plowed, grows rapidly, while tobacco following a good bluegrass sod in an old cultivated field may develop severe root-rot injury. The disease as it occurs in Kentucky has been called brown root-rot because it was believed to be similar to brown root-rot as it occurs in Connecticut, Maryland, Wisconsin, and Ontario.

At the Tobacco Disease Council meeting held at Tifton, Georgia, in the summer of 1946, meadow nematodes were discussed as a cause of injury to tobacco roots, by Jenkins and Graham. Technics for examination of roots for meadow nematodes and their identification were considered, and some discussion resulted as to the possible relation of meadow nematodes to brown root-rot. That meadow nematodes have been almost completely neglected in this country as a cause of root disease was brought out by Steiner in 1945.[2] In the spring of 1945 Doctor Steiner, in conversation, suggested that meadow nematodes might be the cause of brown root-rot in tobacco.

Following the Tifton meeting an examination was made of roots of tobacco plants from the Station brown root-rot plots at Lexington. Roots of several Burley varieties of tobacco were infested with what appeared to be the meadow nematode, *Pratylenchus pratensis*. Examinations were then made of tobacco roots from 132 other plots in the tobacco rotation and fertilizer series in all of which tobacco has started slowly in past years. Meadow nematodes were found in every sample collected.

Washed roots were kept overnight in a Petri dish of water. In the morning the roots were removed from the dish and the nematodes picked

from the bottom of the dish with the aid of a stereoscopic microscope. At first the nematodes were removed with a bamboo pick, but later much more efficiently with a capillary pipette into which a nematode was drawn very quickly if the tip of the pipette was placed near it. Final examination was made with a compound microscope. Nematodes with a spear in the mouth parts were considered to belong to the meadow nematode group. It was not unusual to find several hundred meadow nematodes in the bottom of a dish in which a collection of tobacco roots had been left overnight.

Examinations were also made by placing small rootlets on a microscope slide in a small amount of water and, under a microscope, gently scraping the small brown lesions with a small scalpel until the nematodes emerged. Many meadow nematodes were removed from small brown lesions on the roots in this way.

These observations demonstrated that in the brown root-rot plots and in other plots in the rotation series where tobacco had started slowly, meadow nematodes were present in large numbers in washed tobacco roots, where they had apparently caused small brown lesions in the cortex. There is no reason, therefore, to believe that brown root-rot as it occurs in Kentucky is different from what is called meadow nematode root-rot in the flue-cured tobacco areas of Georgia, the Carolinas, and Virginia.

There is also reason to believe that brown root-rot as it occurs in Ontario, Canada is caused by the same agent that causes the disease in Kentucky. We have been studying the relative resistance of varieties of Burley tobacco to brown root-rot in the field for several years. The trials have included the Burley varieties Canada 354, susceptible; Canada 364, resistant; and Harrow Velvet, highly susceptible in Ontario to brown root-rot. The tests also included Ky. 33 and a local Kentucky variety, Canadian; both are resistant to black root-rot and resistant to brown root-rot at Lexington, Kentucky. These varieties were sent to Ontario for trial in brown root-rot plots. The five Burley varieties, including two susceptible and three resistant ones, gave the same reactions to brown root-rot at the two locations, strongly suggesting that the cause of brown root-rot is the same in both areas.

From a study of the literature on brown root-rot as it occurs in Wisconsin, Maryland, and other northern states, it is probable that the meadow nematode is concerned in brown root-rot in those areas also.

GROWTH OF TOBACCO UNDER DIFFERENT SYSTEMS

In contrast with the slow growth of tobacco following sod crops on the rotation series is the rapid growth, following setting, of tobacco in an old bluegrass field that had not previously been plowed in the history of the Kentucky Experiment Station. This field had been lightly pastured and had a heavy bluegrass sod. A small area is plowed each year for tobacco. The tobacco starts quickly, grows rapidly, and produces about 2000 lb. an acre. Twenty-seven collections of tobacco roots from this field were made; 16 were free from, 8 had a total of 42, and 3 a total of 302 meadow nematodes.

These results suggested that the nematodes present were limited to small areas. In the black root-rot plot and in 2 other plots tobacco is grown every year, with a small grain or a grain and hairy vetch cover crop. Although the fields are heavily infested with *Thielaviopsis basicola*, varieties highly resistant to this organism start growth quickly following transplanting and grow rapidly throughout the summer. Seven collections of tobacco roots from these fields gave only two meadow nematodes, while four collections of tobacco roots from the nearby brown root-rot plot yielded 1894 meadow nematodes. Fifteen collections of vetch roots during late fall and winter from the continuous tobacco fields had a total of only 62 meadow nematodes, and 14 collections of barley roots from the same fields gave a total of 26. One collection of vetch from the brown root-rot area nearby had 357, another vetch collection from the brown root-rot area had 992, while a barley collection from the same plot gave 199 meadow nematodes.

The system of continuous culture to tobacco does not appear to be favorable to the meadow nematodes under Kentucky climatic conditions, while long grass rotations with tobacco only occasionally seem to be very favorable to them. Burley tobacco growers in increasing numbers are growing tobacco year after year on the same land with a cover crop of a small grain and hairy vetch turned under about 3 weeks before transplanting tobacco. This system has resulted generally in tobacco that starts growth quickly following transplanting, grows uniformly and rapidly throughout the summer, and shows no evidence, from the standpoint of growth, of brown root-rot injury. In contrast, fields following bluegrass sod, alfalfa, lespedeza, orchard grass, and other pasture mixtures frequently show distinct evidence of brown root-rot, and in the few cases studied meadow nematodes have been present in the roots.

DISTRIBUTION OF MEADOW NEMATODES

No general survey of meadow nematodes in Kentucky has been attempted, but in the course of the present studies enough evidence has been gained to show that they are by no means limited to the Experiment Station plots. They have been found in tobacco roots from Simpson, Fayette, Henry, Boone, Owsley, Taylor, Clark, Warren, and Montgomery counties; in the roots of grass and clover from Bourbon, Scott, Woodford, Fayette, and Boone counties; in the roots of *Nicotiana rustica* from Jefferson County; and in tobacco roots from Ohio County, Indiana. These results suggest a rather general distribution in pasture and forage crop roots.

MEADOW NEMATODES IN THE ROOTS OF OTHER CROP PLANTS

It has been pointed out repeatedly by others that brown root-rot is severe following certain grass crops. If meadow nematodes are the cause of brown root-rot, then one should expect to find the nematodes in the roots of plants that precede a brown-root-rot-affected crop of tobacco and not in the roots of plants preceding a healthy crop. Filipjev[3] and Stekhoven include species

---

[3] Filipjev, I. N., and J. H. S. Stekhoven, Jr. A manual of agricultural helminthology. I-XV, 878 pp. E. J. Brill, Leiden. 1941.

in 34 plant families in the host range of *Pratylenchus pratensis*. In the present studies we have examined the roots of most of the plant species growing in 118 of 338 plots, in the tobacco rotation series, not in tobacco in 1946 but to be planted to tobacco in 1947. Grass and legume crops on this series of plots have never been so luxuriant as would be expected, considering the fertility of the soil. Roots from one plot only were without meadow nematodes, indicating that practically the whole series of plots is infested.

Meadow nematodes apparently identical with those found in tobacco roots were present, often in large numbers, in the washed roots of orchard grass, crab grass, bluegrass, timothy, redtop, fescue, and corn; in red clover, lespedeza, alfalfa, sweet clover, and soybeans; and in the weeds, *Sida spinosa*, *Oxalis sp.*, *Cyperus esculentus*, dandelion, *Plantago lanceolata*, dock, *Erigeron canadensis*, *Rumex acetocella*, knotweed, pigweed, *Solanum carolinense*, *Physalis sp.*, and in *Lactuca scariola*, in which they are unusually abundant. Ragweed roots from plots where other plant roots were heavily invaded yield only an occasional meadow nematode. According to Lunn and associates[4] tobacco makes excellent growth following a crop of ragweed as compared with that following other weeds and natural weed fallow. With heavy meadow nematode infestation of grass roots and roots of other perennials in the fall and winter, it would be expected that abundant invasion of newly developing tobacco roots would take place following transplanting because the roots of the tobacco plants are set directly in the upturned roots of the preceding crop.

DISCUSSION

The results of the present study have shown that meadow nematodes are abundant in the roots of tobacco plants affected by what we have called brown root-rot; and that the roots of crop plants that precede tobacco and are known to produce conditions favorable for the development of brown root-rot are also heavily invaded with meadow nematodes in an area where brown root-rot occurs every year in tobacco. In other fields on the Station farm where tobacco starts growth quickly following transplanting, meadow nematodes have either not been found or found in very small numbers. While final proof is not presented that brown root-rot is actually caused by meadow nematodes and by nothing else, at least there is good evidence that the nematodes cause extensive injury to the roots of tobacco and of several other crop plants, and that this injury is of the type usually described for brown root-rot. It is also evident that meadow nematodes must be given serious consideration as a cause of failure or partial failure of other crops, as the small grains, corn, grasses, and legumes in Kentucky and neighboring states.

Kentucky Agricultural Experiment Station,
Lexington, Kentucky.

[4] Lunn, W. M., D. E. Brown, J. E. McMurtrey, Jr., and W. W. Garner. Tobacco following bare and natural weed fallow and pure stands of certain weeds. Jour. Agr. Res. [U.S.] 59: 829–845. 1939.

REPORT AND ABSTRACTS OF THE TWENTY–NINTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 29th annual meeting of the Pacific Division of the American Phytopathological Society was held in San Diego, California, June 17–19, in conjunction with the meetings of the Pacific Division of the American Association for the Advancement of Science. Approximately 60 persons were in attendance from Arizona, British Columbia, California, Nevada, Utah, Washington, and Territory of Hawaii.   June 17 and 18 were devoted to presentation of papers, 2 short business meetings, and a symposium on ''Fungus Root Diseases of Crop Plants,'' with an all-day field trip June 19.   Twenty-three formal papers were presented in addition to 10 presented in the symposium.   George W. Fischer asked to be relieved of his duties as secretary-treasurer and his resignation was accepted.

The following new officers were elected for 1948:

President: L. C. Cochran
Vice President: George W. Fischer
Secretary-Treasurer: George A. Zentmeyer

Nineteen abstracts were submitted:

Disinfecting power of propylene oxide and propylene chloride in relation to phytopathogenic bacteria and fungi.   PETER A. ARK. Propylene oxide gas at a concentration of 0.3 cc./l. inactivated *Xanthomonas phaseoli, Pseudomonas medicaginis phaseolicola, Ascochyta pisi,* and *Fusarium solani* f. *cucurbitae* in Petri dishes in 45 minutes under vacuum, at 25° C., while it required 0.5 cc./l. to kill *Verticillium sp.* during the same period of time.   *Agrobacterium tumefaciens* and *Corynebacterium michiganense* are killed only beginning at 0.5 cc./l. of propylene oxide with an exposure of 60 minutes.   Concentration of 1 cc./l. for 10 minutes, was lethal to all test organisms mentioned above. Propylene chloride was found to be lethal with concentrations beginning at 0.4 cc./l., with a minimum exposure of 12 hours. Higher concentrations shorten the period of exposure somewhat. No difference in dosages was observed whether the tests were performed *in vacuo* or not. Club wheat seed loses about 50 per cent in germination when treated by propylene chloride, 1 cc./l., under vacuum for 6 hours. No loss in germination was observed at 4-hour exposure and 1 cc./l. also under vacuum.

Effect of crystalline streptomycin on phytopathogenic bacteria and fungi.   PETER A. ARK. Streptomycin (crystalline base) has been found to be toxic to fourteen species of phytopathogenic bacteria, both Gram positive and Gram negative. *Agrobacterium tumefaciens* had a considerable resistance to it. *Actinomyces scabies* is very susceptible to streptomycin, but *Fusarium solani* f. *cucurbitae, Phytophthora capsici,* and *Ascochyta pisi* were not affected by it. Cucumber, tomato, barley, oat, and sunflower seeds soaked 24 hr. in a streptomycin solution, as strong as 200 units per ml. showed no detectable injury and grew normally when planted in soil in a greenhouse. Cucumber seeds artificially contaminated with *Pseudomonas lachrymans,* and treated for 20 min. with the streptomycin solution containing 100 units per ml. were freed of the pathogen. Potato and carrot slices soaked in the streptomycin overnight and inoculated with *Erwinia carotovora* failed to develop soft rot. Development of *Erw. carotovora* was stopped when potato and carrot slices were inoculated and treated with streptomycin after intervals of 20 to 120 min.   The results strongly suggest the possibility of using streptomycin against seed-borne phytopathogenic bacteria.

Stemphylium leaf spot of China aster.   KENNETH F. BAKER and LILY H. CLARK. An unreported disease of China aster caused localized serious losses in cutflower fields near Redondo Beach, California, in mid-summer, 1946. The growers had saved seed from infected fields. Brown, circular or irregular coalescent spots, 1–10 mm. across developed abundantly on leaves, calyces, petals, and sometimes stems. *Stemphylium botryosum* Wallr. *sensu* Wiltshire sporulated on the spots, and its pathogenicity was demonstrated. Infection occurs through stomata and requires high humidity for 2 days; the spores proliferate for 24 hr., rupturing their walls before forming germ tubes. Conspicuous spots develop in 5 days. The fungus is seed borne and, during epigeal germination, sporulates on the seed coats, infecting the young plants. The disease is restricted to fields watered by overhead sprinklers in low areas protected from the drying effect of wind. This limitation is explained by the prolonged spore germination period. The disease should be controlled by using ditch irrigation and avoiding such low, humid areas. The fungus is distinct from *Alternaria zinniae, A. tenuis,* and the Alternarias reported as

Macrosporium florigenum and *M. caudatum* on China aster elsewhere. *Stemphylium botryosum* has been reported on China aster seed in Denmark.

The incidence and development of lemon shell bark. E. C. CALAVAN. Two types of pre-lesion symptoms of shell bark have been found about midway through the bark of mature Eureka and Lisbon lemon trees. Brown necrotic spots impregnated with a hard gummous substance, from 0.5 mm. to 3 cm. long, commonly precede "slow" shell bark lesions. "Fast," rapidly spreading lesions, however, frequently originate over thin tangential plates of discolored phloem. Considerable gum appears to be within the cells of those plates. Streaks of discolored phloem extend vertically from a plate ahead of the growing lesion. New lesions generally form during a flush of growth, particularly from February to May, and crack several months later. The first lesions of shell bark usually appear on the portion of the trunk having the thickest bark, commonly the south or east side just above the bud union. Fungi are apparently of little importance in the incidence of shell bark, but may accelerate lesion development. Numerous species of fungi have been isolated from the margins of advancing lesions, but no fungi have been found in 700 pre-lesions. Shell bark probably is due either to a virus infection or to physiogenetic factors.

Powdery mildew on sugar beet. EUBANKS CARSNER. Powdery mildew on sugar beet was observed in a few fields in the Yakima Valley near Toppenish, Washington, in the summer of 1945 and more extensively in that same valley in the summer of 1946. In the second season a small infestation was also noted near Nyssa, Oregon. In some cases, infected beet leaves showed a color reaction but no evidence was observed of serious injury. The perfect stage of the fungus has not been discovered.

Studies on the natural spread of the peach-mosaic virus among apricots, almonds, and peaches. L. C. COCHRAN and GILBERT L. STOUT. Early in the investigations of the peach-mosaic disease in Southern California, apricots and almonds were recognized as naturally affected hosts. Surveys showed a high percentage of affected trees in apricot orchards adjoining mosaic affected peach and old peach orchard sites, with less or none where peach has not been present. In a 500-tree experimental orchard of alternating peach and apricot planted alongside an old highly infected apricot orchard near which all other peach was previously removed only two peach and no apricot trees became infected during ten growing seasons. Similar rare cases have developed in several grower's peach orchards under parallel conditions. In another peach orchard approximately one mile distant from the experimental block, in which mosaic trees were allowed to stand, surveys by control agencies showed an annual increase, totaling 37.8 per cent during ten years. If the few cases of mosaic occurring in peach planted near mosaic affected apricots and almonds represent natural spread from these hosts, the rate is extremely slow in contrast to spread from peach to peach. No converse data were obtained but the apparent correlation of high incidence in apricot and proximity to mosaic in peach indicates rapid spread from peach to apricot.

Wood pocket, a newly reported disease of lemons. H. S. FAWCETT and E. C. CALAVAN. Wood pocket (ligno—cortosis) was first noted in 1937 in one strain of Lisbon lemons at the Citrus Experiment Station, Riverside, California. A defect or break in the bark is accompanied by discolored wood underneath. Later discolored regions are seen in a tangential cut as an irregular pattern of gum-filled tissue, darkly dotted on a lighter colored surface. The lesions vary from an inch in length to large lesions extending several feet along one side of the larger branches. Few to many small lenticular yellow to ocherous spots, averaging 0.5 to 1.5 mm., in the cambium of the trunk or branches is the first interior symptom. Trees propagated in 1939 by buds from apparently healthy branches of diseased trees developed wood pocket in 6 years. These, when top-worked to various cultivars in 1946, have transmitted the leaf symptoms to healthy scions of several varieties of lemon. Some leaves have a variegated chimera-like aspect, with broad bands in shades of green to yellow to white or lace-like reticulations involving a part or the entire blade. Seedlings from seeds of mature lemons from diseased trees showed some plants with these leaf symptoms. The occurrence of leaf symptoms in seedlings, coupled with the transmission into healthy scions, indicates the presence of a virus.

Multiple sex factors in Ustilago striiformis f. hordei. GEORGE W. FISCHER. The sex reaction was studied of 46 monosporidial cultures of *Ustilago striiformis* forma *hordei,* taken from 12 collections of the stripe smut on species of *Agropyron* and *Elymus,* mostly from the western states. Previous investigations of the forma *hordei,* based on cultures from a single collection, had revealed only 2 sex groups. In the present work, only 2 groups were noted within any one collection of smut, whether or not the monosporidial cultures came from the same or different chlamydospores. When all 46 cultures

were paired up in all possible combinations on water agar, however, it was discovered that 6 distinct sex or compatibility groups were represented.

Some studies of curly top of flax. N. J. GIDDINGS. Specimens of diseased flax received from the southern San Joaquin Valley during the spring of 1945 were found to be infected with the curly-top virus. Since that time 37 species and varieties of flax have been grown in the greenhouse and tested to learn if they showed any differences in reaction to the various strains of curly-top virus. A few of the tests are incomplete at this time, but all commercial varieties have been susceptible to infection by each of the virus strains used and to severe injury by the more virulent strains. Some varieties have shown less injury than others as a result of infection and it is possible that further tests will give more evidence of such differences. The least amount of injury was induced by virus strains 7, 2, and 4 while strains 5, 6, 9, and 3 were highly virulent and resulted in a high mortality among the plants of most varieties. *Linum lewisii* Pursh. secured from Theodore Payne, seedsman, appears to be highly resistant. *Linum perenne* and *L. flavum*, secured from Aggler and Musser Seed Company, also appear to be very resistant, while *L. grandiflorum rubrum* from the same source does not. The experiments thus far have not given any indication of flax species or varieties which would be of help in differentiating curly-top virus strains.

The development of Pierce's disease and its occurrence in rogued and nonrogued vineyard plots. WM. B. HEWITT. Though the occurrence of Pierce's disease varied in different districts of the San Joaquin Valley, the general development and spread of the disease increased very rapidly after 1934 and reached a peak about 1941. The annual incidence has since declined in similar proportions. The occurrence of Pierce's disease in a vineyard, a district, and even the entire valley followed three general patterns; 1, irregularly scattered over most of the area; 2, centered in small localized areas; and 3, concentrated in portions of vineyards adjacent to alfalfa or irrigated pastures. The systematic removal of diseased vines twice each season, once in the spring and again in the fall, in vineyard plots varying in size from 10 to 120 acres each, did not significantly influence the occurrence of new cases of disease when compared with similar vineyards that were not so rogued. Also the annual incidence of Pierce's disease was not apparently influenced in two additional 10-acre plots where the diseased vines were removed several times each season over a period of five years.

Control of brown rot and oleocellosis of citrus fruit in the packinghouse. L. J. KLOTZ and G. A. ZENTMYER. If brown rot infection of lemons (incubation at 60°–65° F.) took place more than 10 hr. previous to immersion in cold fungicides the decay was unchecked. If the period of incubation (60° F.) was 30 hr. or less brown rot could be stopped by 4-min. immersion in water at 120° F. To endure the hot immersion without liberating rind oil and suffering the surface breakdown called oleocellosis, lemons must be slightly wilted by letting them stand 3 to 7 days after picking. Storing lemons at 40 per cent relative humidity, 70°–75° F., in air with rapid movement did not adequately protect them from rind oil spotting during subsequent immersion at 120° F. Exposing inoculated lemons to hot moist air (100°–104° F., 95 per cent R.H.) not only stopped brown rot even after 36-hr. incubation at 53° F. but conditioned the fruit so that no rind oil was liberated during the hot immersion. Lemon oil allowed to remain one or more seconds in contact with the surface of silver or green lemons and then removed with warm soda ash-soap solution caused definite injury.

Effects of curly-top virus strains on extent of injury in root tips of susceptible and resistant sugar beets. C. F. LACKEY. Three strains of curly-top virus were used. Strain 1, a virulent one, causes severe vein roughening and leaf distortion on susceptible beets; strain 2, a less virulent one, produces very little dwarfing and only mild vein roughening of susceptible beets; strain 6 produces very little vein roughening or leaf distortion but does greatly dwarf susceptible beets and causes death in a high percentage of cases. Susceptible beet root tips infected with strain 1 show marked degeneration and necrosis of the cells surrounding the sieve tubes. The resistant beet tips show only an occasional cell with some degeneration. Strain 2 produces only mild degeneration in a few of the cells surrounding sieve tubes and this degeneration is about equal in amount in root tips of susceptible and resistant beets. Strain 6 apparently damages susceptible beets by extending injury to their root tips rather than to their leaves, as is true of strain 1. The severe degeneration and necrosis it causes involves many other cells besides those surrounding the sieve tubes. The injury in the root tips of resistant beets is of the same type as in those of susceptible beets but less severe.

Comparative growth in culture of eleven physiologic races of Ustilago bullata. J. P. MEINERS: In an effort to determine the effect of various temperatures on the cultural

characteristics of head smut of grasses (*Ustilago bullata*), monosporidial lines of 11 physiologic races were grown for 4 weeks in cultural chambers at a uniform relative humidity of 81 per cent and at constant temperatures of 5, 10, 15, 20, 25, and 30° C. Each of the 11 races was represented in the study by 2 monosporidial lines of opposite sex derived from the same chlamydospore. In general, the results showed that not only does temperature influence rate of growth tremendously, but also that the individual races apparently vary in their cardinal temperatures. The optimum temperatures for cultural growth of most races were at 20° and 25° C. Some races grew well over a wide range of temperatures, whereas others appear to be adapted to a narrower range. Color and topography varied greatly with changes in temperature. Monosporidial lines from the same chlamydospore but of different sex reacted alike to changes in temperature.

Effect of daylight on volatile toxicity of lime-sulphur to fungi. JOHN I. MIRHIJ. Turbidity of 0.05 to 0.5 per cent of lime-sulphur increased with increasing light intensities. Hydrogen sulphide evolution was greater in dark than in light from 0.01 per cent lime-sulphur. Germination of conidia of barley mildew (*Erysiphe graminis*) was almost completely inhibited in 5 hours in vapors from 0.1 per cent lime-sulphur in daylight, but inhibition was less in darkness, and less with increasing or decreasing lime-sulphur concentrations, except for those of 0.001 to 0.01 per cent, where inhibition was greater in darkness; and for concentrations of 10 to 100 per cent lime-sulphur, where light had no effect on toxicity. For bean leaves infected with rust (*Uromyces phaseoli*) for 4–5 days before treatment, the same relations with respect to light and lime-sulphur solution applied. Hydrogen sulphide produced from ferrous sulphide and sulphuric acid was more toxic to bean rust and more injurious to bean leaves in light than in darkness, while chemically produced sulphur dioxide was more toxic to bean rust in darkness. Complete eradication of bean rust without injury was obtained from hydrogen sulphide but not from sulphur dioxide.

An indicator agar for the determination of the relative concentration of ascorbic acid in potato tuber tissue. WM. NEWTON. An indicator agar that reveals the approximate concentration of ascorbic acid in potato tuber tissue by direct contact is prepared by heating together 2 gm. agar, 75 ml. water, 10 ml. of a 10 per cent solution of potassium iodide, and 5 ml. of a 1 per cent solution of soluble starch. After cooling to 60° C., 5 ml. of glacial acetic acid and 5 ml. of an 0.01 N solution of potassium iodate are added and the mix is immediately poured into Petri dishes. When uniform filter-paper discs saturated with standard ascorbic acid solutions or uniform tuber-tissue discs are placed upon the agar surface, after 24 hr. at 5° C. the size of the area decolorized is directly related to the concentration of ascorbic acid in the solutions and tissue discs. Tissue discs from tubers affected with mosaic and leaf roll decolorize a greater area than discs from normal tubers from the same variety, and the decolorization is independent of tuber size. The diagnostic accuracy of the method is further strengthened by the tendency of virus infected tissue to remain white and healthy tissue to become blue-black.

Fungi causing root rots of cereals in California. JOHN W. OSWALD. Isolations over a six-year period have shown that eight pathogenic fungi are involved in the cereal root rot complex in California. The disease has been found in 114 fields representing 16 counties. Widespread on wheat and barley in the order of their apparent importance are *Helminthosporium sativum, Fusarium graminearum (Gibberella saubinetii), Fusarium culmorum, Ophiobolus graminis,* and *Fusarium nivale (Calonectria graminicola). Pythium graminicolum* and *Sclerotium rolfsii* were found in local areas on barley, and *Helminthosporium avenae* similarly on oats. The seedling blight and adult root rot phases of the five common pathogens are severe on wheat and barley, whereas oats have proven tolerant to all but *F. culmorum. H. sativum* and *F. graminearum* are frequently found together in the same field and often in the same host. Perithecia of *F. graminearum* have been observed in the field only once, their failure of development probably being due to low humidity when temperatures are sufficiently high. The absence of wheat or barley scab in the State is explained by lack of ascospore inoculum and inadequate humidity at heading time. *Wojnowicia graminis, Fusarium equiseti,* and *F. scirpi,* though commonly associated with the disease, have proven to be only of secondary importance.

Similarities in the pathological anatomy of Quick-Decline- and Tristeza-diseased orange trees. HENRY SCHNEIDER, A. A. BITANCOURT, and VICTORIA ROSSETTI. Radial sections of the phloem at the bud union in mature trees suffering from the two diseases have been compared. In the early stage of both diseases, the sieve tubes and companion cells below the union became necrotic and occasionally parenchyma cells became hypertrophied. In later stages the older sieve tubes above the union were also necrotic. Intensified cambial activity on the phloem side at the bud union resulted in a bulge on the

cambial face of the bark in advanced stages of the disease. Such hyperplastic tissue was similar to normal phloem; but the cells were smaller in size, and the sieve tubes became necrotic on reaching maturity or in some cases at a later date. New sieve tubes below the union less often became necrotic than those in the vicinity of the union, and no hyperplasia occurred there. Occasionally parenchyma cells above the union divided to produce a callus-like tissue. No differences in the anatomy of the two diseases were noted.

Rhizoctonia solani on field crops in the West. RODERICK SPRAGUE. Nearly 4 per cent of all fungi isolated from the roots of Gramineae in the northern part of the western United States were *Rhizoctonia solani.* Symptoms included seed, root, stem, and culm rots. Inoculations in the greenhouse at Mandan, North Dakota, and at Pullman, Washington, during 1940 to 1947 inclusive disclosed that five races of this species were recognizable by differences in their attacks on Kubanka wheat, Marion oats, blue grama, crested wheatgrass, Turghai proso millet, black amber sorghum, and Grimm alfalfa grown at 50°–70° F. Race 1 was virtually nonparasitic. Race 2 was weakly parasitic on alfalfa and proso but caused up to 90 per cent loss in blue grama and up to 16 per cent loss in oats and wheat. Race 3 was similar in reaction but could cause 50 per cent loss in sorghum and complete loss in alfalfa. Race 4, which was common on range grasses, especially bluegrasses, was highly pathogenic to all of the indicator plants, causing a seed and root rot. Race 5, present in the coast region of Oregon and Washington, was also parasitic on all these hosts except alfalfa, which was highly resistant; but this race caused a culm rot after emergence.

Variations in response of fungi to fungicidal chemicals. GEORGE A. ZENTMYER and L. J. KLOTZ. The fungicidal efficiency of ·25 organic chemicals was tested *in vitro* against diverse types of fungi. Closely related compounds differed greatly in effectiveness; 8-hydroxyquinoline, a metal-precipitating chemical, was highly fungistatic, while its isomer, 2-hydroxyquinoline, which does not react with metals, was low in toxicity to fungi. The fungicidal action of p-nitrophenol and o-nitrophenol paralleled the action of these chemicals in inhibiting polyphenol oxidase in higher plants. The action of p-nitrophenol against fungi may be the result of a similar enzyme inhibition. Marked specificity was noted in the action of some organic chemicals, including diphenyl, benzoic acid, Dithane (disodium ethylene bisdithiocarbamate), catechol alpha-terpineol, ethylene dibromide, Dowfume N (mixture of dichloropropane, dichloropropene). Such cosmopolitan saprophytes or facultative pathogens as *Trichoderma lignorum* and *Botryosphaeria ribis* (*Dothiorella gregaria*) were generally highly resistant to most chemicals tested. These fungi are evidently able either to tolerate or metabolize a wide variety of chemicals, as would be expected from their growth on diverse substrata. The age of culture used in mycelial transfers markedly affected the response of several fungi to fungicidal chemicals; transfers from young cultures (1 day old) were more resistant to fungicides than transfers from older cultures. Variations in effectiveness of fungicides on different culture media were also noted.

REPORT AND ABSTRACTS OF THE FIRST ANNUAL MEETING OF THE NORTHEASTERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The Northeastern Division held a very successful initial meeting at Amherst, Massachusetts, on November 26 and 27, 1946. This meeting was held in conjunction with the New England-New York Spray Specialists' Conference. Plant pathologists from all the Northeastern States and from different fields of interest comprised the 75 in attendance. Consequently there was active participation in the discussion meetings on Apple Scab, New Fungicides, Potato Diseases, and Dutch Elm Disease. Twelve formal papers were presented at one afternoon session. At the short business meeting the following officers were elected for 1947: President, S. E. A. McCallan; Vice-President, M. T. Hilborn; Secretary-Treasurer, W. T. Schroeder; Councilor, Thomas Sproston, Jr.

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Nematodes on Tobacco in Connecticut. ANDERSON, P. J. Nematodes, serious pests of tobacco in the South, have not previously been reported on tobacco in Connecticut. In the summer of 1946 a severe infestation of the root-knot nematode (*Heterodera marioni*) caused severe stunting of shade tobacco in one field in Windsor. On other fields two other species of nematodes were found associated with deterioration of the roots and stunted growth of the plants. One of these was identified as the meadow nematode (*Pratylenchus pratensis*). How widespread nematode infestation may be in the tobacco fields of New England is not known because (1) a survey has not been made, and (2) nematode damage may have been attributed to other disease organisms.

Control of Tobacco Mildew. ANDERSON, P. J. Dusting tobacco seed beds with a 20–80 Fermate (ferric dimethyl dithiocarbamate) dust gave as complete control of mildew as did the standard Fermate spray, 1–50. Two bismuth subsalicylate spray preparations gave equally good control. The protective action of the bismuth preparations did not last longer than that of Fermate.

The Interference Phenomenon Between the Rib-Grass and Tobacco-Mosaic Viruses in Bean. BEALE, HELEN PURDY. When the rib-grass virus (*Marmor tabaci* H. var. *plantaginis* H.) is rubbed on the leaves of Early Golden Cluster bean (*Phaseolus vulgaris* L.), no systemic infection results and no local necrotic lesions occur, such symptoms that are characteristic of tobacco-mosaic virus (*Marmor tabaci* H.) on this host. In spite of the noninfectiousness of the rib-grass virus to Early Golden Cluster bean, whenever this virus is mixed in increasing concentration with relatively smaller quantities of tobacco-mosaic virus, the number of local lesions capable of resulting from the inoculation of tobacco virus alone is successively reduced. Because of this interference phenomenon existing between the two viruses, a preparation of rib-grass may contain a trace of tobacco-mosaic virus which is nondetectable by inoculation of bean, the usual criterion for freedom from contamination with tobacco virus. If the sample of rib-grass virus to be tested is inoculated first in tobacco (*Nicotiana tabacum* L.), in which both viruses multiply systemically, a sufficiently high concentration of tobacco virus will be produced to permit detection upon subsequent inoculation of bean although only a trace may be present in the original sample.

Performance of New Fungicides for Control of Potato Late Blight. DAVIDSON, R. S. and A. E. RICH. Comparative studies on the performance of some new organic fungicides for the control of potato late blight have been conducted at the Rhode Island Agricultural Experiment Station during 1945 and 1946. In 1945, 12 different synthetic organic materials were compared with Bordeaux. None of the sprays effected as complete control as did Bordeaux. Phygon (2,3-dichloro 1,4-napthoquinone), Fermate (ferric dimethyl dithiocarbamate), Dithane 14 (disodium ethylene bisdithiocarbamate), and Zerlate (zinc dimethyl dithiocarbamate) were significantly better than the check treatments. Fungicidal properties of 7 organic materials were further investigated in 1946. Again, Bordeaux proved superior to all other chemicals when applied as foliage sprays. However, Zerlate, Phygon, and G–11 (di-trichloro-dihydroxy diphenyl methane) were significantly better than

the check treatments. It was observed in both seasons that materials which effected significant control over the checks produced less injury to the foliage than did the copper containing sprays. The failure of these organic sprays to control late blight apparently was due in part to their poor adherence. In 1945 and 1946 DDT was applied in all treatments, and in 1946 a control fungicide plot was included which received only DDT. By comparison of a control which received neither a fungicide nor an insecticide with the control which received only DDT, it was evident that DDT did not possess fungicidal properties, while G–11, which is similar chemically to DDT, proved to have fair fungicidal properties.

Oxyquinoline Benzoate Aids Suppression of Symptoms of Dutch Elm Disease. DIMOND, ALBERT E. Late in July, 200 elms up to 6-inch diameter were inoculated basally with giant spore loads of *Graphium ulmi*. At this time half the trees were watered with 1: 1000 oxyquinoline benzoate and half with water. On August 20, foliage symptoms in the crown averaged 47.1 per cent for checks and 29.4 per cent for treated trees, a highly significant difference. During September, check trees dropped leaves earlier than treated ones. Three per cent of treated and 5 per cent of check trees failed to develop symptoms. Cultures were recovered from all but 14 per cent of treated and 5 per cent of check trees. Some of these showed no symptoms. Effects were not of practical magnitude in this experiment, but the method of inoculation was much more severe than in nature. Wilted diseased trees to which oxyquinoline benzoate was applied recovered from wilt, and appearance of further symptoms was arrested. Apparently fewer trees succumb to the disease if treated and more trees recover when treated with oxyquinoline benzoate. As shown by culture recovery, this treatment does not kill the fungus in the diseased tree.

Fungicides Applied in Fertilizer for the Control of Cabbage Clubroot and Damping-off. DORAN, W. L. Fungicides were mixed with a 5: 8: 7 commercial fertilizer and this was applied to soil at the rate of 15.6 gm. of the fertilizer per square foot immediately before seeding. The method is convenient; especially in the case of an application too light to be distributed evenly unless in some carrier. Dithane D–14 (disodium ethylene bisdithiocarbamate) and Dow Seed Protectant No. 9 (zinc trichlorophenate) thus applied controlled damping-off better or with less injury than when applied in water immediately after seeding. Mercurous chloride and other mercury salts (0.15 or 0.20 gm. per square foot) controlled clubroot better and more safely in soils which had previously received an application of hydrated lime (20.0 gm.) or sodium chloride (10.0 gm. per square foot) than they did in untreated soil. And Dithane was more effective against damping-off in a limed soil. But the fungicides named above, as well as Fermate (ferric dimethyl dithiocarbamate), Phygon (2,3-dichloro 1,4-naphthoquinone), and zinc mercaptobenzothiazole, were all less effective against clubroot than was Tuads (tetramethyl thiuram disulfide). Tuads, 0.55 gm. per square foot, applied in fertilizer, gave good control of clubroot and of damping-off and markedly improved the growth of cabbage seedlings in both limed and unlimed soils.

Eradicating Apple Foliage Scab With Summer Sprays. GUBA, E. F. The action of summer eradicant sprays on a block of 19-year-old McIntosh trees has been studied. Following applications, mass spore germination tests were made at frequent intervals to determine the action of the sprays. The trees were sprayed at 450-lb. pressure, and 15 to 16 gallons were applied per tree. Liquid lime-sulfur (1–50) gave fair results. Two to three applications almost completely eradicate scab. The treatments are accompanied by more or less injury to the fruit and foliage. Puratized Agricultural Spray (phenylmercuri triethanol ammonium chloride) (½ pint–50 gal. water) operates to kill the incubating and sporulating scab fungus and to stop infection completely without any apparent injury to the foliage. Both fungicides cause the collapse of the conidia and change the color of their contents. The conidia are inactivated completely following applications of Fermate (ferric dimethyl dithiocarbamate) (¾ lb.–50 gal. water) without accompanying changes in structure or color of the spores. Negligible spore germination was maintained throughout the season by 6 applications beginning at calyx on May 17 in one set of trees, and 3 applications beginning on June 10 in another set of trees. The character of the scab mold on the foliage appears to remain healthy, suggesting fungistatic rather than fungitoxic action. The action of Phygon (2,3-dichloro 1,4-napthoquinone) (¾ lb.–50 gal. water) was somewhat comparable with that of Fermate although inactivation was not so complete. Wettable sulfurs lack scab eradicant action. None of the materials eradicated fruit scab lesions.

The Characteristic Curve for the Action of Copper Sulphate on the Germination of Spores of Sclerotinia fructicola and Alternaria oleracea. McCALLAN, S. E. A. Extensive data were obtained for the dosage response curve of copper sulphate on spore germination inhibition of *Sclerotinia fructicola* and *Alternaria oleracea*. Thirty-four doses, from 0.105 to 32.000 p.p.m. Cu, with a dose ratio of $\sqrt[4]{2}$ were used, 1000 spores counted at each dose and the entire test run 3 times, giving about 100,000 spores for determining the characteristic curve. Elaborate precautions are necessary for defining germination and ascertaining prior germination and nongermination of control spores. The final percentage

inhibition of germination for treated spores is derived from the formula $100 - \dfrac{100\ (T-P)}{C-P}$ where C and T are percentage observed germination in controls and in treated spores, respectively, and P is percentage of prior germination in control. Smooth curves concave upward are obtained on logarithmic probability paper. By means of Parker-Rhodes α transformation straight lines result for the regression of probits on conc. α. For $S.$ $fructicola$ $\alpha = 0.41$ and $E = 1.354\ X + 0.590$, for $A.$ $oleraceae$ $\alpha = 0.56$ and $E = 0.525\ X + 1.873$. However, a negative α transformation is required to straighten convex curves. The Langmuir adsorption equation when applied above gives concave curves. Germ tube length expressed as percentage of control plotted against percentage germination gives a sigmoid curve.

1946 Potato Spray and Dust Experiments in New York State. NIEDERHAUSER, J. S., W. A. RAWLINS, and A. M. FRENCH. Seven large-scale experiments comparing potato fungicides in combination with standard amounts of DDT were conducted in the important potato-growing areas of New York State. Dithane (D–14) (disodium ethylene bisdithiocarbamate) ranked first in three of four spray experiments, and Dithane (HE–178) was first in two of three dust experiments. The yields obtained with fixed coppers were about equal to those obtained with Bordeaux mixture spray or copper lime dust. Zerlate (zinc dimethyl dithiocarbamate) failed to control late blight in the one test where it was included. However, late blight was not an important factor affecting yield in any of these experiments, and appeared late in the season if at all. In a replicated experiment covering 30 acres of Chippewa potatoes, airplane dusting did as well as ground dusting in late blight and insect control. Lower yields from ground-dusted fields were attributed directly to wheel-row damage. Detailed studies on distribution of the dust applied at 40 lb. an acre and in a 30-foot airplane swath showed 4.7 milligrams of copper deposited per square foot directly under the plane, and 2.9 milligrams of copper deposited per square foot at a distance of 15 feet from the center of the swath.

Some Problems Involved in Use of 2,4-D as a Tomato Defoliant. SCHROEDER, W. T., and F. G. SMITH. Further field trials confirmed the previous year's results that 2,4-D reduced the cracking of ripe fruit occurring in periods of excessive precipitation. The amount of cracking was inversely proportional to the extent of defoliation or vine damage caused by 2,4-D and dependent upon its time of application. Results also showed that 2,4-D treatments may affect quality and yield of the product in other ways. Processed juice from treated fruit was inferior in flavor and color. The percentage of anthracnose was increased, especially in the absence of an adequate protective fungicide schedule. From the standpoint of avoiding frost damage, even the earliest applications of 2,4-D did not significantly increase the rate of ripening as determined by yield. In fact, these applications caused lower yields, probably by reducing fruit size. However, in excessively wet seasons, control of cracking and resulting fruit mold might offset this reduction in yield. These results emphasized the complexity of problems involved in 2,4-D treatment of tomatoes and suggest that other defoliants be similarly investigated.

PHYTOPATHOLOGICAL NOTES

A Chromatographic Method for the Detection of Tobacco-Mosaic Virus in Juice from Diseased Turkish Tobacco Plants.—A simple paper-chromatographic method has been developed for the detection of tobacco-mosaic virus in extracted juice of Turkish tobacco plants. A chromatographic method using paper has been described by Consden, Gordon, and Martin[1] for the separation of mixtures of amino acids. It has been modified somewhat in testing for tobacco-mosaic virus.

The apparatus used is shown in figure 1. A glass cylinder (1) is used as a support for a large rubber or cork stopper (2) on which is placed a porcelain embedding dish (3). Glass microscope slides (4) are inserted in cuts made in the stopper on each side of the dish to support the paper strips (5). Whatman no. 1 filter paper, 2×8 inches, is used in the test. This apparatus is placed in a large glass jar fitted with a tight cover. A small amount of water is placed in the bottom of the jar to give a saturated atmosphere for the tests.

Several color reactions were tried experimentally with purified tobacco-mosaic virus. The Sakaguchi[2] arginine reaction appeared to be the most sensitive color test for the virus on Whatman no. 1 filter paper. The reagents for the test are used in the following order: *1*) 10 per cent aqueous potassium hydroxide; *2*) 0.1 per cent alpha naphthol in 50 per cent ethyl alcohol; *3*) 5.25 per cent sodium hypochlorite (undiluted Chlorox). When these reagents are applied to the filter paper by means of an atomizer, presence of arginine is indicated by the appearance of a bright pink color in about one minute. The color remains for several minutes and then fades.

Since arginine is also present in normal plant proteins, the success of the test depends upon the ability of the solvent system to move the virus to a given spot on the paper strip while not moving normal arginine-containing proteins to that same spot. To make the test a drop of plant juice is placed $1\frac{1}{4}$ inches below the top of the paper strip. Juice from plants known to be healthy may be placed on the same paper strip and used as a control. The paper is folded at a point 1 inch below the top. It is hung on the edge of the glass slide with the shorter portion extending into the solvent dish and the longer portion hanging freely in the atmosphere of the jar. When the solvent is added to the dish it is taken up rapidly by the paper. If the proper solvent is used the virus will be moved to a new location by the liquid as it passes down the paper. When the advancing front of the solvent approaches the bottom of the paper, the paper strip is removed and dried. The paper is then treated with the arginine reagents to indicate the position of the virus.

[1] Consden, R., A. H. Gordon, and A. J. P. Martin. Qualitative analysis of proteins. A partition chromatographic method using paper. Biochem. Jour. 38: 224–232. 1944.

[2] Sakaguchi, S. A new color reaction of protein and arginine. Jour. Biochem. (Japan) 5: 25–31. 1925.

It was found that water buffered at a pH level of 4 or lower would not move the virus while water buffered at a pH level of 4.5 or higher moved tobacco-mosaic virus to give a satisfactory color test. When buffers having a pH level of 6 to 7 were used as solvents, very satisfactory color tests were obtained and the transported virus was recovered in a highly active form. The best recovery procedure was to run two strips simultaneously under the
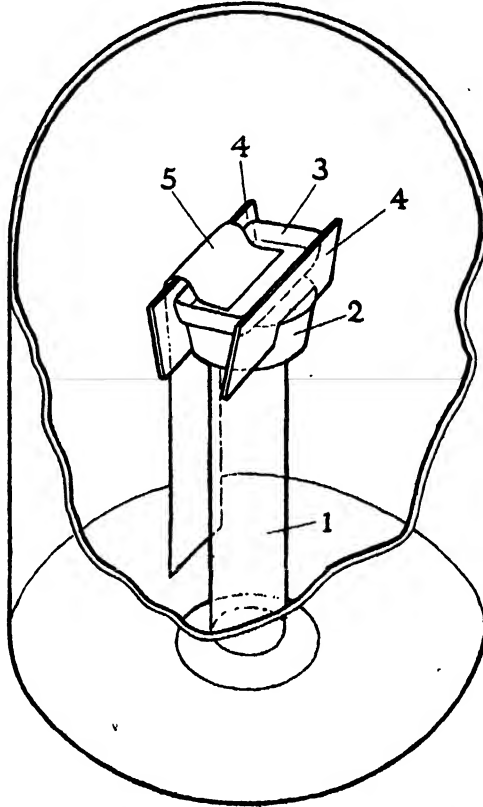
FIG. 1. Cut-away view of large glass jar showing the apparatus used for the test. A glass cylinder (1) supports a large rubber or cork stopper (2), on which is placed a porcelain embedding dish (3). Glass microscope slides (4), inserted in cuts made in the stopper, support the paper testing strip (5). (Drawing by J. A. Carlile.)

same conditions. The position of the virus was located by applying the color reagents to one of the strips. The virus location was then outlined with pencil on the other strip. The spot was cut out and the virus was removed from the paper by elution with buffer. When water buffered at a pH level of 4.5 or higher was used as the solvent system, most of the normal arginine-containing proteins and the chlorophyll remained at the original site of application and did not interfere with the color test for the virus.— G. W. COCHRAN, Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, New Jersey.

The Fungicidal Value of Mixtures of Lime Sulphur and Zinc Sulphate.—The discovery by Heuberger[1] that the addition of zinc sulphate increases the fungicidal value of disodium ethylene bisdithiocarbamate suggested that a similar effect might result if zinc sulphate is added to lime sulphur. Such a mixture is already in successful use as a combined insecticide and zinc deficiency spray[2] and it is apparently a more efficient insecticide than the same mixture without zinc.

A series of concentrations of zinc sulphate was added to a series of concentrations of lime sulphur and the mixtures tested as eradicant and protective sprays on a number of plants and diseases by methods previously described.[3] Data were plotted as lime-sulphur dosage against percentage
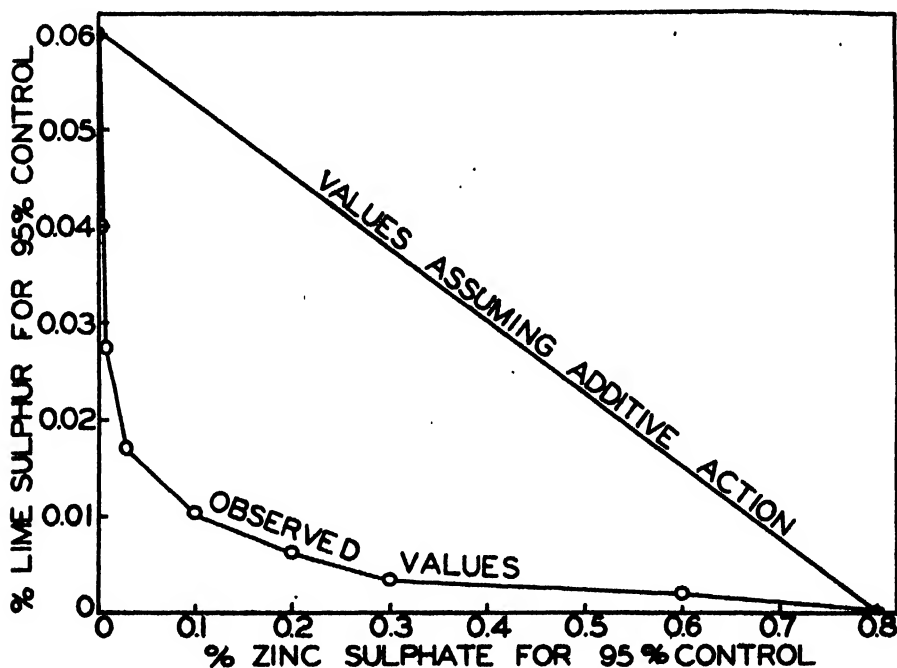
FIG. 1. The protective value for bean rust of mixtures of lime sulphur and zinc sulphate.

of disease control, and the dosage of lime sulphur for 95 per cent control with each concentration of zinc sulphate was determined. The greatest synergism observed was in greenhouse tests with bean rust, and average results of 15 trials are summarized in figure 1. In these tests the LD 95 for lime sulphur alone was 0.06 per cent concentration of applied spray. The addition of zinc sulphate decreased the LD 95 at all concentrations

[1] Heuberger, J. W. Factorial studies on Dithane plus zinc sulphate-lime: "the reaction product" zinc ethylene bisdithiocarbamate. Phytopath 36: 685–686. 1946.

[2] Lewis, H. C. Spray injury from zinc–lime sulphur in Central California. Calif. Citrograph 31: 112. 1946.

[3] Yarwood, C. E. The function of lime and host leaves in the action of Bordeaux mixture. Phytopath 33: 1146–1156. 1943.

tested. With 0.1 per cent zinc sulphate the LD 95 was 0.01 per cent lime sulphur, or about one sixth that required when lime sulphur was used alone or one fifth that expected of the combination if additive action is assumed. Other uses in which mixtures of zinc sulphate and lime sulphur have been more fungicidal than expected on the basis of additive action were as eradicants for bean rust, as protectants against bean powdery mildew, as protectants against hop downy mildew, and as protectants against snap-dragon rust, though in all these cases the synergistic effect was less than in the case of bean rust.

Iron sulphate and copper sulphate were also tested separately as supplements to lime sulphur and disodium ethylene bisdithiocarbamate. All combinations were synergistic for bean rust but none so greatly synergistic as zinc sulphate with these same fungicides. As protectants and eradicants for bean and cucumber powdery mildews, however, the iron sulphate-lime sulphur combination may have been as effective as the zinc sulphate-lime sulphur mixture.—C. E. YARWOOD, University of California, Berkeley, California.

TEMPERATURE REQUIREMENTS FOR GERMINATION OF SPORES OF CRONARTIUM FUSIFORME

PAUL V. SIGGERS[1]

In the southern half of the Gulf States the pycnial and aecial stages of *Cronartium fusiforme* (Arth. & Kern) Hedge. & Hunt, the cause of fusiform rust, most commonly occur on elongated cankers on woody parts of loblolly pine (*Pinus taeda* L.) and slash pine (*P. caribaea* Morel.), and less frequently on longleaf pine (*P. palustris* Mill.). The uredial and telial stages occur on the leaves of oaks, chiefly species of the pointed-leaf or black oak group.

This disease has become increasingly prevalent in the lower Gulf region during the last 35 years. Since loblolly and slash pines are less resistant than longleaf pine to infection, wider distribution and intensification of the disease may be ascribed in part to the natural encroachment of the more rust-susceptible pines and oaks on lands originally occupied almost exclusively by longleaf pine.

In recent years the reforestation program for the lower South created a strong demand for slash pine planting stock (8). By 1940, the estimated annual production of slash pine at seven nurseries, exclusive of planting stock produced at two U. S. Forest Service nurseries, was 37 million seedlings, compared with 11,000,000 loblolly pine and 2,600,000 longleaf pine seedlings. Since slash pine is highly rust-susceptible, a problem of controlling the fusiform rust soon developed in southern pine tree nurseries. Sleeth (9) reported that losses of slash and loblolly pine seedlings, caused by the disease, exceeded 4,000,000 in 1938 and amounted to 3,000,000 in 1939.

Bordeaux spray treatments to control the disease have not given consistently favorable results. Control measures at one nursery showed little worthwhile benefit in 1939 and 1941. Results of the treatments were favorable in 1940 (9) but less favorable in 1942.

Knowledge of environmental factors affecting rust infection is of fundamental importance in control of the disease in forest tree nurseries. For example, spore germination is necessary for infection, therefore, a better understanding of the effect of temperature on germination may aid in the development of more effective spray schedules for control of the disease in nurseries. Knowledge of the temperatures that inhibit spore germination would be of great aid in studying and interpreting the results of inoculations. The effect of temperature on spore germination was studied during the spring and fall of 1945 and during the spring of 1946.

METHODS AND MATERIALS

In all tests spores were germinated in direct contact with distilled water or in a moisture saturated atmosphere. Van Tieghem cells were used in

germination tests of aeciospores, urediospores, and teliospores. Two cells were prepared for each glass slide, by fastening glass rings (3 mm. high by 15 mm. diameter) to the slide with high-melting-point paraffin. As an added precaution against loss of water, the Van Tieghem cells were sometimes placed in Petri dishes lined with moist filter paper. In some tests the cells were placed inside large iceless refrigerators (5), instead of Petri dishes. Sporidia were germinated in water under cover slips on glass slides.

Spore Germination Technique

Different methods were devised for germinating different types of spores. With aeciospores and urediospores, a drop of water was centered on a cover slip and a small fragment from a young oak leaf was placed on top of the water. A small quantity of spores was transferred to the drop by the point of a sterile inoculating needle. The cover slip bearing the droplet was then quickly inverted over the ring. The spores floated more or less freely on the surface of the water.

The telium was the unit of observation in germination tests involving teliospores, because teliospores cannot be separated from the telium-matrix and still remain viable. A drop of water was placed in the bottom of the Van Tieghem cell. Oblong sections of oak leaves, with telia, were cut slightly longer than the diameter of the ring so that the leaf segment, when mounted in the cell, would arch above the water with the telia pointing downward over the drop. A glass cover slip closing the cell prevented excessive loss of water.

Sporidia were obtained by placing telia in Van Tieghem cells overnight at temperatures near the optimum for teliospore germination. In the morning, after the sporidia had been formed, telia were cut off and mounted on a glass slide in a drop of water under a cover slip. The slides were then tapped lightly to detach mature sporidia from the promycelia.

Duration of Germination Tests

Germination tests with aeciospores, urediospores, and teliospores started in late afternoon, usually about 5 p.m. and continued for 15 hours. Germination tally of aeciospores and urediospores, starting the following morning, continued sometimes until the 16th hour. One hundred to 150 spores were counted in each test. Germination of sporidia lasted for 5 or 6 hours; however, 3 tests ran 9 hours. Sixty to 100 spores were counted in tests with sporidia.

Temperature Equipment

For low-temperature tests an electric refrigerator was available and for high-temperature germination an electric incubator was used. For intermediate temperatures, home-made iceless refrigerators were constructed and mounted in shallow vats filled with water and kept moist by water dripping from above. Maximum and minimum thermometers recorded the range of temperatures in tests within the electrical refrigerator and in-

cubator. A Friez hygrothermograph recorded humidity and temperatures within the iceless refrigerator. The accuracy of this instrument was checked about once a week with a Friez psychrometer.

The refrigerator and the incubator could not be classed as biological constant-temperature equipment and there was no temperature control inside the iceless refrigerators. Temperatures within the humidity chambers varied with that on the outside, except that during the warmer periods of the day it was usually 3° to 7° C. cooler inside the chambers than outside.

Source of Spores Used and Locality of Collection

Aeciospores came from 6 fusiform cankers on *Pinus caribaea* and *P. taeda* from Harrison, Jones, and Stone Counties in southeastern Mississippi and from *P. rigida*[2] Mill., Bent Creek, N. C.

Urediospores were obtained by inoculating leaves of seedlings of *Quercus nigra* L. and from 2 field collections on *Q. phellos* L. near Pearlington, Mississippi, dated March 24, 1945.[3]

Teliospores originated from leaves of seedlings of *Quercus nigra* that had been inoculated with aeciospores of *Cronartium fusiforme*.

Sporidia came from teliospores that, in turn, had resulted from inoculation of leaves of *Quercus nigra*. All inoculations were made in a greenhouse near Saucier, Miss.

PRELIMINARY EXPERIMENTS

Preparatory to study of the effect of temperature on germination of rust spores, the minimum period required to start germination and the germinative capacity with reference to age of spore collection and storage conditions were investigated. There was no information available on the time required to initiate spore germination, and observations in the field indicated that the yellow-ochre color associated with fresh aeciospores disappeared in about 5 weeks. When this color change occurred it was thought that the spores were no longer viable.

Minimum Period Required to Start Germination

Aeciospores. In five tests with as many different collections of aeciospores, the minimum period required for the germination of aeciospores varied from 2¼ hours to about 4 hours (Table 1).

Urediospores. The minimum period for the initiation of germination was determined for two lots of urediospores. A day-old collection of spores from greenhouse inoculation of a water oak (*Quercus nigra*) seedling was

[3] Urediospores from natural infection were resorted to because uredial sori were seldom produced abundantly by inoculation. The writer believes that the uredial sori collected in March, 1945 were those of *Cronartium fusiforme* because the ranges of *Pinus echinata* Mill. and *P. virginiana* Mill., the more common hosts for *C. cerebrum* (Peck) Hedge. and Long (3) do not extend as far south as Pearlington, Miss.; more than 99 per cent of the cankers in the locality of collection were fusiform, in contrast to the globose canker formed by *C. cerebrum*, and also *C. fusiforme* fruits earlier in the growing season than *C. cerebrum*. Peak development of the uredial stage took place earlier in 1945 than in any other year of record.

incubated at 25°–26° C. Between 6 and 7 per cent of the spores germinated in 1 hour. Spores from a 7-day collection on willow oak (*Q. phellos*) started germination after incubation at 22°–23° C. for 1 1/3 hours.

Teliospores. Nongerminated teliospores of 29-day-old telia[4] from greenhouse inoculation were held at temperatures fluctuating from 17°–23° C. and examined at 3-hour intervals starting 9 a.m. April 19, 1945. Microscopic examination at 6 p.m. showed teliospores were germinating but not abundantly. Under these conditions, nine hours was the minimum period for germination of teliospores. By 9:15 p.m. of the same day teliospores were germinating from the tip to the base of the telium and sporidial "spore prints" (Fig. 1, A) on the surface of the drop of water contained clusters of 50 to 150 sporidia. Obviously, abjection of sporidia had set in just a short time before.

The effect of shorter exposures to temperature and moisture conditions favorable for teliospore germination was studied. Teliospores of 17-day-

TABLE 1.—*Minimum period required for germination of aeciospores of Cronartium fusiforme*

Source of spores	Age of collection (Days)	Temperature (°C.)	Duration (Hours)	Results
Pinus taeda	37	23	1¾	Negative
P. caribaea	36	21–23	2½	Negative
P. rigida	23	23	2¼	Positive—1 pct.
P. caribaea	51	23	3¾	Positive—trace
P. taeda	44	23	5¾	Positive—5 pct. germinating between 3¾ and 5¾ hrs.

old telia were exposed to favorable temperature and moisture conditions for periods of less than 9 hours on 3 successive days and stored at night in a refrigerator, at 11°–15ª C. Under these conditions, a small number of sporidia were found at the end of the third day after a cumulative exposure of 23 hours to favorable environmental conditions.

Sporidia. Sporidia obtained in May from 14-day-old telia and incubated for 3 hours at 17°–18° C. started germination. Sporidia from the same source, incubated at 21°–22° C., started germinating in 2½ hours. Twenty-eight per cent of the spores had germinated by the fourth hour. In a third test, with telia about 38 days old, sporidia incubated at 28°–29° C. started germination in 2¼ hours. Seven per cent of these spores had germinated by the fifth hour.

Germinative Capacity with Reference to Age of Collection and Storage Conditions

Aeciospores. Aeciospores from a canker on slash pine, collected March

[4] The age of telia in days was counted starting from the first day that primary sori from greenhouse inoculations became visible to the unaided eye. After inoculation with aeciospores in mass, the shortest incubation period was found to be 6 days, yet for about 10 days thereafter additional telial sori—also primary sori as they resulted from a single application of aeciospores—showed up on the same leaf.

14, 1945 and stored below 10° C., maintained germinative capacity at high level for 47 days. Aeciospores from loblolly pine, collected the same day and stored under similar conditions, maintained good viability for 76 days. Spores from this lot germinated (0.5 per cent) on October 8 and were used successfully for inoculation on October 12, 211 days after collection. Some lots of aeciospores remain viable for months, at low temperatures, but
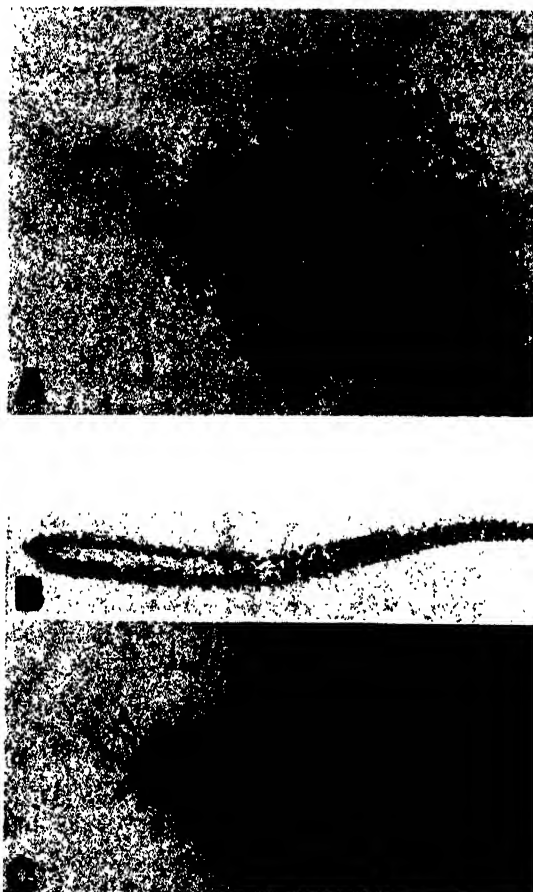
FIG. 1.   Sporidia and telia of *Cronartium fusiforme*: A. Sporidial spore print formed from telial column held in a moist chamber for 15 hours; approximately 1600 sporidia in the cluster that was formed on a droplet of water.   × 65.   B. Telium with nongerminated teliospores, obtained by inoculation of oak leaves with aeciospores on April 30, 1945. Photographed June 14, 1945.   × 45.   C. Apical end of a telium with germinating teliospores.   × 85.

germinative capacity is reduced as the storage period lengthens. The first change in color of aeciospores, from yellow-ochre to gray, was found to be associated with a loss in germinative capacity.

Urediospores. Five per cent germination was obtained with urediospores collected March 24, 1945 and stored at 4° to 10° C., when tested on October 5, 1945. These were germinated after 15 hours exposure to 17°–22° C. The

same spore collection was used successfully for inoculation on Nov. 2, 1945, 223 days after collection.

Teliospores. Although dormant teliospores (Fig. 1, B) withstand unfavorable environmental conditions, germinative capacity is reduced if the telia are subjected to high temperatures that approach lethal temperatures. Teliospores of 29-day-old telia formed a few sporidia in one of two Van Tieghem cells on June 7, 1945. In the next test, on June 9, teliospores failed to germinate after 23 hours at 23°–24° C. In a final test, started June 12 at 6 p.m. and lasting 70 hours, with the temperature at 23°–24° C., sporidia were deposited in one of four cells. This indicates that the fungus was still alive in one of the leaf segments and that young teliospores, developing at the base of the telial column, had germinated during the relatively long period of exposure at favorable environmental conditions. Probably most of the fungus in the leaf tissue had been killed by high temperatures and low humidities that prevailed prior to the test. Daily maximum greenhouse temperatures exceeded 32° C. during the week starting June 3 and exceeded 35° C. during the following week.

Sporidia. Attempts to store sporidia were not successful. All observations indicate that sporidia are short-lived, and that high temperatures or moisture conditions unfavorable for germination quickly cause loss of germinative capacity.

THERMAL LIMITS AND OPTIMUM TEMPERATURES FOR GERMINATION

Since the spore germination data presented were based on fluctuating temperatures, the mean temperature was determined for each test from the range. Most observations were made with Fahrenheit thermometers but, for convenience, the data are presented in the Centigrade scale. The thermal limits and optimum temperatures recorded for spore germination, although not shown in table 2, were obtained from graphs based on the Fahrenheit scale. In converting to degrees Centigrade, fractions in °C. were rounded off to the nearest whole number.

Aeciospores. Eighty-two tests were run with aeciospores from several sources. The lower and upper thermal limits for germination are slightly below 11° C. and 29° C. The optimum temperature for germination is about 21° C. Good germination occurred over a range of average temperatures from 17° C. to 22° C. (Table 2).

Urediospores. The results from 54 germination tests with urediospores are given in table 2. The lower and upper thermal limits for germination were found to be slightly above 8° C. and at 29° C., respectively. The optimum temperature for germination is about 18° C. The data (Table 2) indicate that urediospores germinated well over a range of average temperatures from 15° to 20° C. Urediospores germinate at lower temperatures than aeciospores and have a lower optimum temperature.

Teliospores. It was not possible to express the germination of teliospores on a percentage basis. Germination failed in 8 tests below 15° C. and in 7 tests above 26° C. The optimum temperature for germination, though

not determined, is thought to be about 21° C. The optimum temperature for the germination of teliospores would be that temperature which induces germination (Fig. 1, C) and formation of sporidia in the shortest time. Teliospores started germination in a minimum of nine hours in weak diffuse light with temperature fluctuating between 17° and 23° C.; however, observations on this point were too few to permit drawing any conclusions.

Sporidia. The lower limit for germination of sporidia was defined by the failure of spores to germinate at an average temperature of 13° C. and 4 per cent germination at an average temperature slightly below 14° C. The

TABLE 2.—*Germination of aeciospores, urediospores, and sporidia of Cronartium fusiforme at different average temperatures*

Temperature class centers	Aeciospores			Urediospores			Sporidia		
	No. of tests	Mean germination	Range in germination between tests	No. of tests	Mean germination	Range in germination between tests	No. of tests	Mean germination	Range in germination between tests
°C.		Per cent	Per cent		Per cent	Per cent		Per cent	Per cent
7	1	0.0	... ...	2	0.0			....	. . ..
9	6	0.0	. . . .	7	6.7	3–11	1	0.0	... ...
11	5	2.7	0–10	1	43.0	.. ..	1	0.0	. . . .
13	8	18.6	3–65	1	12.0	.. ..	3	2.6	0–4
15	5	65.8	31–86	5	75.0	32–90	..	... ...	....
17	10	67.7	40–91	5	85.4	73–92	1	61.0	. . ...
19	9	76.8	61–98	4	76.7	73–82	9	60.1	28–80
21	9	83.1	53–97	7	75.0	60–98	4	69.2	48–92
23	6	73.3	49–95	10	56.7	25–84	6	78.1	46–93
25	6	43.5	15–69	6	34.0	19–54	4	44.0	13–82
27	10	9.2	1–17	3	2.3	0–4	7	17.7	4–50
29	7	2.9	0–9	2	3.0	0–6	6	5.0	3–9
31	.....	....	....	1	0.0	... ..	.	. . ...	... ...
Total	82	. ..	... .	54	.. .	. .	42	.	.. ..

upper limit for germination appears to be a little above 29° C. average temperature. The optimum temperature for germination is 22° C.

DISCUSSION

The minimum average temperature for germination of aeciospores was slightly below 11° C. and the maximum average temperature was 29° C. Doran (2) and Hirt (4) working with aeciospores of *Cronartium ribicola* Fisch. v. Waldh. found the lower and upper thermal limits for germination to be 5° C. and 28° C., respectively. Germination of fusiform rust spores above 27° C. is chiefly of academic interest because in the Gulf region outdoor temperatures above 27° C. are always associated with periods of relatively low humidity—too low to permit germination of rust spores. Furthermore, reduced hyphal growth and apical curling of the germ tubes were commonly observed in tests in which the average temperatures ranged from 26° to 29° C. Inoculation tests confirmed the results from germination

studies. In 14 successful inoculations, temperatures never exceeded 26° C. during the first 48 hours, except in one test 27° C. was recorded between the 17th and 18th hours. This was followed by a steady drop to 18° C. during the next 17 hours. Although aeciospores and urediospores of *C. fusiforme* germinated at 27° C. it seems probable that infection would not occur at temperatures maintained at 27° C.

The study indicated that teliospores withstand temperatures that would be lethal to other types of spores. For example, exposure of aeciospores to 27° C. for 15 hours reduced germination to 1 per cent; thereafter, only 1 per cent germinated when exposed to lower laboratory temperatures for 24 hours. In a parallel test at 19° C., 98 per cent germinated in 15 hours. On the other hand, teliospores that were formed March 6, 1945, and were exposed to 29°–34° C. for 5½ hours on May 1, 1945 and to 27°–32° C. on May 7, germinated and produced sporidia on May 8.

·Both aeciospores and urediospores germinated in water and it is conceived that an adequate amount of water vapor in the air would permit germination of urediospores. The locally restricted pattern of secondary uredial sori sometimes noted on leaves, extending downward from the vicinity of a primary sorus to lower parts of the same leaf surface, strongly implies that water served to distribute the spores from the primary sorus to lower areas of the leaf and that infection occurred only where the droplet or film of water had been. It is generally known that a practically saturated atmosphere is necessary for germination of teliospores of species of *Puccinia,* as indicated by Clayton (1) and Maneval (7) and the moisture requirements for germination of teliospores of *Cronartium fusiforme* appear to be equally high. Both teliospores and sporidia germinated in moisture-saturated atmosphere, and it is thought that teliospores germinate only in this way. Teliospores of telia immersed in water overnight for 15 hours did not germinate. · Sporidia that had been deposited on a glass slide in an iceless refrigerator became plasmolyzed a few minutes after they had been removed to relatively dry atmosphere in an open greenhouse.

Spring spores produced by *Cronartium fusiforme* develop relatively early in the season and are ready to germinate very soon after they are formed. Aeciospores, for example, may infect as early as February. Near Pearlington, Hancock Co., Miss., telial columns about 1 mm. long were found March 15, 1944 on leaves of an oak growing beside a loblolly pine with numerous fruiting fusiform cankers. Inasmuch as the minimum period from inoculation to the appearance of primary telial sori is 6 days, at best, and the average daily growth of a telium is about 100 μ, these telia originated from infection that occurred late in February.[5] Uredial sori appear most abundantly in early April. Primary telial sori may appear two or three days after the first uredial sori. Field observations by Sleeth (9) and by

[5] Teliospores developed and germinated in a minimum of 8 days from inoculation of oak·leaves with aeciospores.⁵ Most of the inoculations resulted directly in telial sori. Retention of oak seedlings in weak diffuse light in iceless refrigerators probably affected photosynthetic activity adversely, resulting in near suppression of the uredial generation (10).

the writer, in the region where *C. fusiforme* is most abundant, indicate that maximum production of sporidia by this species of rust takes place in April or in May, and that infection, as a seasonal process, is essentially completed by the latter part of June.

In the infection of pines, the moisture-temperature-time relationship involved in the germination of teliospores, the discharge of sporidia, and their germination are significant. With favorable temperature and moisture conditions, 9 hours are required to start germination of teliospores, 3 hours are needed for development of sporidia, and 6 hours elapse before the abjected sporidia germinate in quantity. In other words, starting with nongerminated teliospores, a minimum of 18 hours, with temperatures fluctuating between 16° and 26° C. and humidity maintained close to the moisture-saturation point are needed for abundant infection on pines.

In nature, such conditions are rather infrequently encountered. The spring of 1938, however, was an unusually favorable season for rust infection. The average percentage of cankered slash pines in 6 of 7 nurseries, from surveys made by Lamb and Sleeth (6), was greater in 1938 than in 1937 or in 1939—usually several times greater. Meteorological records from a weather station in Harrison County, Mississippi, indicate that eight times, between March 28 and May 8, 1938, temperature and moisture conditions favorable to sporidial production and germination prevailed for 18 hours or longer. At no time in 1937, and only once in 1939 did similar conditions prevail during the same period. This explains why the bulk of natural rust infections appear to be confined to certain years, during which the conditions favoring production, dissemination, and germination of inoculum briefly but frequently prevailed, resulting in relatively heavy infection in those years.

Control measures in forest tree nurseries where the fusiform rust has consistently caused loss of planting stock should be timed with reference to periods when the infection hazard is high. In nature, sporidia are produced in large numbers, if prolonged dry weather in April and May is followed by a period of rainy weather for a day or more. Spraying a day before a rainy period would prove more effective than application of a fungicide 3 or 4 days before the humid period. Timing the treatments in this manner should be feasible, with long-range weather forecasts available. Since germination of sporidia, and, therefore, infection of pine seedlings does not occur at 13° C., spraying could be deferred as long as the daily average temperature is not in excess of 13° C.

SUMMARY

The effect of temperature on germination of four types of spores produced in the spring by *Cronartium fusiforme* (Arth. & Kern) Hedgc. & Hunt, the cause of fusiform rust, has been studied. In all tests spores were germinated in distilled water mounts or in an atmosphere maintained at the moisture saturation point.

The minimum period required to start germination was investigated preparatory to a more detailed study of the effect of temperature on the process. Fresh aeciospores started germination in 2¼ hours at 23° C. A day-old collection of urediospores started germination in less than an hour at 25° C. Teliospores started to germinate in a minimum of 9 hours at 17° to 23° C., and sporidia, in 2¼ hours at 28° to 29° C.

Germinative capacity of a spore lot depends chiefly on age of the collection and storage conditions. Storage at low temperature, below 10° C., kept germinative capacity of aeciospores at high level for a maximum of 76 days. Urediospores stored under similar conditions were successfully used for inoculation 223 days after collection. Teliospores germinated after exposure to fluctuating temperatures in a greenhouse from March 6 to May 8, 1945. Attempts to store sporidia were not successful.

The lower and upper thermal limits for germination of aeciospores are slightly below 11° C. and 29° C. The optimum for germination is about 21° C. For urediospores, the lower and upper thermal limits for germination are slightly above 8° C. and at 29° C. Urediospores germinate at lower temperatures than aeciospores and have a lower optimum temperature for germination. The lower and upper limits for germination of teliospores are approximately 15° C. and 26° C. The optimum temperature for germination of teliospores was not determined. Sporidia germinate between 13° and 14° C., with the upper limit a little above 29° C. and the optimum temperature at 22° C.

Timing the spray treatments with reference to periods of high infection hazard and deferring control measures as long as daily average temperatures lie below the minimum thermal requirements for germination of sporidia should aid in the preparation of more effective spray schedules for control of the disease in forest tree nurseries.

U. S. DEPARTMENT OF AGRICULTURE

LITERATURE CITED

1. CLAYTON, C. N. The germination of fungous spores in relation to controlled humidity. Phytopath. 32: 921–943. 1942.
2. DORAN, WM. L. The minimum, optimum, and maximum temperatures of spore germination in some Uredinales. Phytopath. 9: 391–402. 1919.
3. HEDGCOCK, G. G., and W. H. LONG. Identity of *Peridermium fusiforme* with *Peridermium cerebrum*. Jour. Agr. Res. [U.S.] 2: 247–250. 1914.
4. HIRT, RAY R. The possibility of Ribes infection by aeciospores of *Cronartium ribicola* at temperatures above 19° C. Phytopath. 27: 104–106. 1937.
5. HUNT, N. REX. The "iceless refrigerator" as an inoculation chamber. Phytopath. 9: 211–212. 1919.
6. LAMB, HOWARD, and BAILEY SLEETH. Distribution and suggested control measures for the southern pine fusiform rust. U. S. Forest Serv., Sou. Forest Expt. Sta. Occas. Paper 91. 5 pp. 1940.
7. MANEVAL, W. E. Germination of teliospores of rusts at Columbia, Missouri. Phytopath. 12: 471–488. 1922.
8. SIGGERS, PAUL V. Fusiform rust: slash pine plantation threat. Miss. Farm Research 9(1): 1, 7. 1946.
9. SLEETH, BAILEY. Fusiform rust control in forest-tree nurseries. Phytopath. 33: 33–44. 1943.
10. WATERS, CHARLES W. The control of teliospore and urediniospore formation by experimental methods. Phytopath. 18: 157–213. 1928.

CORRELATED RESISTANCE OF LEAVES, COTYLEDONS, AND STEMS OF CUCUMIS MELO L. TO CANTALOUPE POWDERY MILDEW (ERYSIPHE CICHORACEARUM DC.)

THOMAS W. WHITAKER[1] AND DEAN E. PRYOR[2]

In a study designed to determine the number and quantitative effects of the genes for resistance to powdery mildew (*Erysiphe cichoracearum* DC.) in *Cucumis melo* L., it has become important to know whether the various aerial organs of the plant (leaves, cotyledons, and stems) are equally susceptible to attacks by the fungus. It is obvious to the experienced observer that in susceptible varieties leaves, cotyledons, and stems are attacked with equal severity. It is also obvious that in strains with a very high level of resistance leaves, cotyledons, and stems are equally resistant to attacks by the fungus. When a cross is made between a very susceptible line and one that is highly resistant, our problem is to determine whether or not the aerial portions of the plants in the F_2, F_3, and subsequent progenies will be attacked uniformly. If the severity of mildew infection is found to be closely correlated on leaves, cotyledons, and stems of an individual, it would seem safe to assume that the same set of genes for susceptibility is effective throughout the entire aerial portion of the plant. However, should the results indicate that the degree of infection on the leaf was not necessarily an indication of infection to be expected on the stems and cotyledons, or *vice versa*, some other explanation would have to be sought. There is the possibility that such behavior might indicate that different organs of the same plant are differentially susceptible to the fungus, or that different biotypes of the fungus exist which attack the several aerial portions of the plant with an unequal measure of success.

EXPERIMENTAL OBSERVATIONS

In order to examine the question of differential susceptibility of the various organs of the same plant, a considerable number of individuals in several F_3 families were scored for resistance to powdery mildew. The reaction of leaves, cotyledons, and stems of each plant was recorded. The technique of inoculation was the same as that used in previous work.[3,4] As soon as the first leaf unfolded, the plants were placed in a glass-sash chamber and inoculated by blowing conidia from heavily infected leaves into the chamber. This method has proved effective in obtaining a uniform, severe infection on all aerial portions of susceptible plants. Disease symptoms were recorded 16 days after inoculation.

A total of eight different F_3 families were examined. The number of individuals scored in each family ranged from a low of 19 up to 175 plants in the largest family. Of the eight F_3 families sampled, two were highly resistant, two were very susceptible, and the remainder were approximately intermediate in their reaction toward the parasite. It is thus evident that a fair sample of the variability to be expected of the segregation from the original cross was tested.

Since the problem to be solved is one of determining to what extent low infectibility (or resistance) of one plant part indicates probable infectibility of another, it seems proper to combine the data from the eight families, in order to obtain r values for the comparisons as a whole.

The scores were converted to an arbitrary numerical scale[5] and the correlation coefficients calculated for leaves and stems, leaves and cotyledons, and cotyledons and stems (Table 1). The significance of the correlation coefficients for the several comparisons was evaluated.

TABLE 1.—*Correlation coefficients for mildew infection on the aerial parts of cantaloupe plants*

	Correlation Coefficients between:		
Number of plants scored	Leaves and stems	Leaves and cotyledons	Cotyledons and stems
859	0.818**	0.838**	0.866**

** Significant at the 1-per cent level.

It is evident from the results that there is a high degree of correlation between the severity of mildew infection on the several aerial portions of the plant.

Since the correlations derived in this study are high, it might be helpful to know whether the examination of the leaves would provide a fairly reliable index of the severity of infection on cotyledons and stems. A value of r for this correlation can be calculated by treating the data in a slightly different manner. Assuming that the best estimate of the severity of infection is obtained from the average of the sum of the effects on leaves, stems, and cotyledons, then the estimate of the severity of infection on the leaves, compared with that from the composite picture, yields a value of $r = 0.940$. Thus about 88 per cent of the possible information ean be obtained by examination of the leaves alone. This means that in most cases severity of infection on the leaves is a good indicator of the mildew symptoms to be found on cotyledons and stems.

[5] In rating the amount of powdery mildew, the following scale has been used. Type 0—No mycelium evident to the naked eye. Type 1—Only 1 to 3 small colonies developed. Type 2—Little mycelium developed and few conidia formed. Type 3—Mycelium sparsely covers part of all of the leaf; sporulation somewhat suppressed (medium infection). Type 4—The mycelium entirely covers the leaf. Sporulation abundant (severe infection). Type A—Used in connection with the several reaction types to indicate stem cracking and necrotic or chlorotic spotting on leaves and cotyledons. In converting to a numerical scale, Type 0 = 1, Type 0A = 2, Type 1 = 3, Type 1A = 4, etc.

If these eight families are analyzed independently, it is found that those with a very high level of resistance are apt to show less correlation of mildew severity symptoms on the several organs than those that are intermediate or susceptible in their reaction.

DISCUSSION AND CONCLUSIONS

The experimental observations lead to the conclusion that there is apt to be a high degree of correlation between the severity of powdery mildew symptoms on the leaves, cotyledons, and stems of the same plant; *i.e.*, moderate infection on the leaves indicates a corresponding degree of severity on cotyledons and stems. The same situation holds with respect to very susceptible leaves and highly resistant ones.

In general, observations of natural infection of cantaloupes with powdery mildew in the field tend to confirm conclusions arrived at on the basis of our greenhouse study. Very susceptible varieties seem to be attacked with equal severity on leaves and stems. However, progenies with a fairly high level of resistance very often produce plants in which the mildew colonies are present only on the leaves. In such cases it is possible that the stems escaped infection, or were infected only lightly, or perhaps became infected at a later date than the leaves.

There is no evidence from these experiments for the presence of more than one biotype of the powdery mildew fungus. Likewise, there is no evidence to indicate that different organs of the same plant are differentially susceptible to attacks by the parasite. The genes for susceptibility seem to be equally effective throughout all the aerial portions of the plant.

UNITED STATES HORTICULTURAL FIELD STATION,
    LA JOLLA, CALIFORNIA.

STEM ROT OF DIEFFENBACHIA PICTA CAUSED BY PHYTOPHTHORA PALMIVORA AND ITS CONTROL

C . M . T O M P K I N S A N D C . M . T U C K E R [1]

INTRODUCTION

Potted plants of two varieties of *Dieffenbachia picta* Schott (the regular or dark green type and one with light green foliage known as Rudolph Roehrs), prized by the florists' trade for their attractive, variegated leaves, have been seriously affected in recent years by a stem rot in commercial greenhouses in San Francisco.

This paper discusses the symptoms of the disease, the causal organism, and recommendations for control.

SYMPTOMS OF THE DISEASE

Under conditions favoring natural infection in local commercial greenhouses, the first visible symptom on young or small, as well as on older or

FIG. 1. Main stems of *Dieffenbachia picta*, grown from stem or cane cuttings: A. Stem showing incipient, water-soaked lesion; B. Advanced stage of infection; C. Cavity formed by collapse of internal tissues; D. Infected stem immediately preceding lodging; E. Healthy stem.

larger plants, consists of a small, irregular-shaped, water-soaked lesion on the main stem at the soil level (Fig. 1, A). Usually the lesion increases in size very rapidly and utimately may extend $\frac{1}{4}$ to 1 inch above and below the

soil level.   As infection spreads internally, the invaded stem tissues become
soft and watery.   Coinciding with infection of the basal part of the main
stem of young plants (arbitrarily, less than ½ inch in diameter), the leaves
and petioles usually turn yellow and wilt very suddenly.   Within a day or
two, the main stem breaks at the point of infection, falls, and the plant dies
(Fig. 4, A, B).   In older or larger plants (whose main stem exceeds ½ inch
in diameter), invaded tissues collapse, forming a brown cavity (Fig. 1,
B, C, D) which, as it continues to enlarge, weakens the structural stability
of the main stem and soon causes breakage and lodging (Fig. 2).   In contrast

FIG. 2.   Stem of an infected *Dieffenbachia picta* plant, grown from a stem or cane
cutting, immediately after lodging.

to young plants, the foliage of older, infected plants does not wilt, either
before or after the main stem collapses.   The leaves and petioles retain their
normal turgidity and color, probably because of the rapid spread of the
disease in a relatively restricted area and the high water content of the
succulent main stem.

Internally, as viewed in longitudinal section, the invaded tissues are
water-soaked to dark gray, and frequently a definite, narrow, black band
separates the diseased from healthy tissues (Fig. 3).

Apparently the roots of diseased plants are not affected (Fig. 2).

The disease is favored by relatively high air temperatures, high humidity, poor soil drainage, excessive irrigation, and crowding of the potted plants on greenhouse benches.

THE CAUSAL FUNGUS, PHYTOPHTHORA PALMIVORA

Numerous tissue plantings from naturally-infected stems of two commercial varieties of *Dieffenbachia picta* were made on malt-extract agar and have consistently yielded a fungus in pure culture which has been identified as *Phytophthora palmivora* Butler.
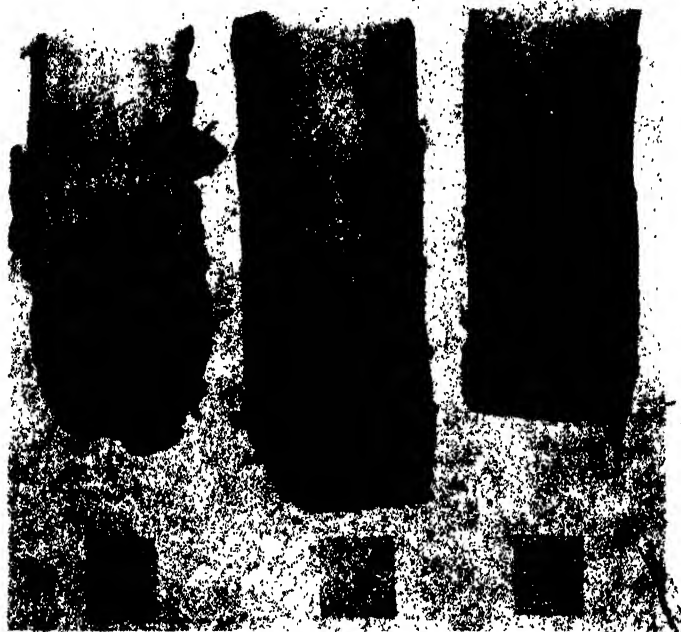
FIG. 3. Longitudinal sections of stems of *Dieffenbachia picta;* A, B. Diseased stems, naturally infected, in advanced stage of decay, showing narrow, black margin adjoining healthy tissues; C. Healthy stem.

Three isolates of the fungus which were studied proved identical. Sporangia and chlamydospores developed abundantly in oatmeal agar cultures, and on tufts of mycelium washed and transferred from pea broth to sterile distilled water.

The sporangia are produced sympodially on slender sporangiophores not inflated or swollen at the nodes and differing but little from the vegetative hyphae. The sporangia are limoniform, with a rounded base, and supported by a pedicel which, on abscission, often remains attached to the sporangium. At the apex the sporangium is provided with a broad, prominent, convex, hyaline papilla. Mature sporangia germinate by one or numerous germ tubes, usually arising adjacent to the papilla, or by the

development of zoospores of the usual Phytophthora type, completely differentiated within the sporangium, and escaping through the orifice resulting from the dissolution or rupture of the papilla. The size of the sporangia varies from 26 to 80 microns in length and 18 to 36 microns in diameter, with a mean size of 48.4 × 27.8 microns.

Chlamydospores are terminal or intercalary, spheroidal, hyaline at first but becoming straw color to brown in older cultures. Considerable thickening of the wall often occurs. Germination is always by germ tubes. The diameter of the chlamydospores ranges from 21 to 51 microns, with a mean of 32.7 microns.

Oogonia and oospores were not observed.
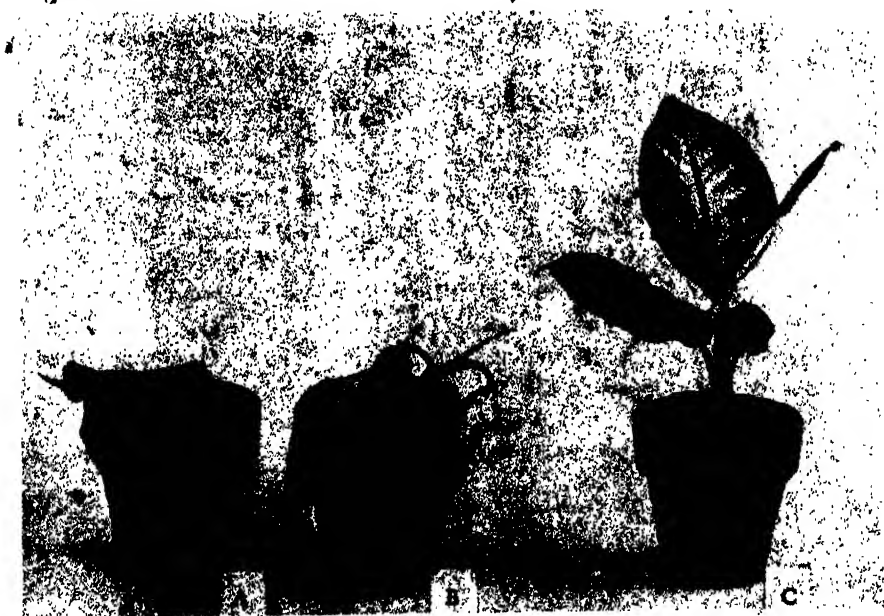
FIG. 4. *Dieffenbachia picta* plants, variety Rudolph Roehrs, grown from stem or cane cuttings. A, B. Diseased plants, showing advanced symptoms, 12 days after inoculation with *Phytophthora palmivora;* C. Healthy control plant.

The fungus corresponds closely to the description of *Phytophthora palmivora* by Butler (3) although he mistakenly regarded the chlamydospores as oogonia. Ashby (1) in 1929 emended the description and established the heterothallic character of the species. He also distinguished isolates of the species as "typical" or "atypical," basing the distinction principally on the amounts of sporulation in culture. The isolates from *Dieffenbachia* may be referred to his "typical" group, producing sporangia and chlamydospores early and profusely.

Isolates of the fungus proved pathogenic to healthy plants of the regular or dark-green, variegated leaf variety of *Dieffenbachia picta* and of the light-green, variegated leaf variety known as Rudolph Roehrs. Inoculum was pre-

pared by growing the fungus on poured plates of malt-extract agar. After 5 days' growth at room temperature, the agar inoculum was cut into ½-inch blocks, the depth of which averaged ⅛ inch. It was added to 6-inch pots of autoclaved soil, each containing a healthy plant, by making a small hole about ½ inch deep at a distance of 1 inch from the main stem, adding 1 or 2 blocks of inoculum, and covering with soil. All pots were heavily watered each day to keep the soil very moist, thus providing optimum conditions for infection. Greenhouse temperatures ranged from 70° to 80° F. while the inoculation tests were in progress. The incubation period ranged from 10 to 15 days for each variety of *Dieffenbachia*, and all infected plants died within 2 or 3 days after the stem and foliage collapsed (Fig. 4). Of 15 plants inoculated, of each variety, all became infected, while the 20 control plants, which had previously been treated by adding blocks of sterile malt-extract agar to the soil, remained healthy. Infected plants had symptoms which were identical with those of naturally-infected plants. The fungus was reisolated from the stems of all infected plants and proved to be identical with the original isolate. The reisolates were highly pathogenic.

The relation of temperature to growth of the mycelium was studied. The culture tubes (2.1 by 20 cm.) used and the procedure followed were those previously described by Tompkins and Gardner (5). The medium used was malt-extract agar, pH 7.0. Inoculated tubes were kept at room temperature for 48 hours. Then 3 tubes of the isolate were placed in a horizontal position in controlled temperature chambers at intervals of 3°, from 4° to 40° C. The cultures were incubated for 96 hours. The cardinal temperatures were determined on the extent of mycelial growth in the culture tubes.

The minimum temperature for growth of the isolate of *Phytophthora palmivora* from *Dieffenbachia picta* was approximately 13° C., the optimum 28°, and the maximum 31°.

Tucker (7), using cornmeal agar with pH 6.2, found that isolates of *Phytophthora palmivora* grew rather profusely at 30° or 32.5° C., but failed to develop at 35°. Of 42 isolates, 4 made no growth at 32.5°, while 38 grew at this temperature, many of them profusely. However, none proved capable of appreciable growth at 35°. Typical and atypical isolates behaved very similarly. The fungus developed most abundantly at 27° to 30°. At the lower temperatures, 1 isolate grew at 5°, 25 at 10°, 15 at 15°, and 2 at 20°. Isolates of the fungus grew most frequently at 10°.

EXPERIMENTAL HOST RANGE

Phytophthora palmivora is a tropical species, characterized by a wide host range. No attempt has been made to review the literature which is scattered and voluminous.

Among species of the Araceae Phytophthoras have been reported on the dasheen (*Caladium Colocasia* (L.) Wight.) and on the calla (*Zantedeschia sp.*). *P. colocasiae* Rac. was described by Raciborski (4) from the dasheen in Java in 1900 and has been identified in numerous Asiatic and Pacific areas.

It is unknown in the Americas.   Infection is usually confined to the leaves
where the fungus causes the development of brown spots.   However, under
favorable conditions invasion of petioles and corms may occur.   On the calla
(*Z. aethiopica* Spreng.) *P. richardiae* Buis. was described in 1927 by
Miss Buisman (2) in Holland as the cause of a root rot.   The disease, or one
with similar symptoms, has been observed in England and at various loca-
tions in the United States.   In 1947 Tompkins and Tucker (6) described a
leaf blight of the pink calla (*Z. rehmannii* Engler) caused by *P. erythro-
septica* Pethyb.   The disease is known only in California.

Studies on the host range of *Phytophthora palmivora* from *Dieffenbachia*
in the greenhouse have been limited to those plants grown most commonly
in the same environment in local greenhouses for the florists' trade.   Em-
ploying the same inoculation technique as heretofore described, 8 plants each
of *Schismatoglottis latifolia* Miq., *Aglaonema simplex* Blume, *A. commu-
tatum* Schott, *Philodendron cordatum* (Vell.) Kunth, *Caladium bicolor*
Vent, tuberous-rooted begonia seedlings (*Begonia tuberhybrida* Voss),
Gloxinia (*Sinningia speciosa* Benth. & Hook.), and pink calla (*Zantedeschia
rehmannii* Engler) were tested.   No infection was obtained, indicating that
the disease under local conditions is confined to the 2 varieties of *Dieffen-
bachia picta* previously mentioned.

CONTROL OF THE DISEASE

Experimental tests conducted in a greenhouse in San Francisco, Cali-
fornia, indicate that this disease can be avoided by rooting stem or cane cut-
tings in 2-inch pots of steam-sterilized sand.   After the formation of a good
root system, requiring from 4 to 5 months, the young plants were transferred
to 6-inch pots of steam-sterilized soil in which they continued healthy as long
as they were held prior to sale.   Cuttings rooted in non-sterile sand and
grown in soil known to be naturally infested with the organism continued to
show a high percentage of diseased plants.

Additional protection against the disease may be provided by dusting the
cuttings with a suitable fungicide.   In tests which were also conducted in
San Francisco, 55 cuttings were dusted with each of the following fungi-
cides: Spergon (mixed with Celite 505 in equal parts by volume), Fermate
(mixed with Celite 505), Arasan, Phygon, and Zerlate.   Controls consisted
of 55 cuttings dusted with Celite 505 and 55 untreated cuttings.   All cut-
tings were then placed in 2-inch pots of steam-sterilized sand on a greenhouse
bench.   After 4 months, examination showed that the cuttings dusted with
Fermate (ferric dimethyldithiocarbamate), Phygon (2,3,dichlor-1,4-naphtho-
quinone), and Spergon (tetrachloro-parabenzoquinone) had developed ex-
cellent root systems and vigorous, healthy foliage.   Less promising results
were obtained with Arasan (tetramethyl-thiuram-disulfide) and Zerlate
(zinc dimethyldithiocarbamate), while the controls yielded the poorest re-
sults.   Apparently the use of certain fungicides as surface dusts on cuttings
of *Dieffenbachia picta* affords protection against infection and stimulates

root and foliage growth. Following the transfer of the rooted cuttings to 6-inch pots of steam-sterilized soil, stem rot has not occurred on any of the plants during the succeeding months.

Well-drained soil and careful watering of the plants in the greenhouse are factors which contribute toward freedom from disease.

SUMMARY

Stem rot of two varieties of *Dieffenbachia picta* occurs in commercial greenhouses in San Francisco, California.

Symptoms of the disease consist of a rapid, wet decay of the stem at the soil level. The foliage of young plants turns yellow and wilts, the top falls, and the plant dies. The foliage of older plants does not wilt but remains turgid and green, both before and after the main stem lodges.

The causal organism has been identified as *Phytophthora palmivora* Butler, on the basis of morphologic characters and temperature-growth relations.

In the greenhouse, infection was obtained by adding the fungus on agar blocks to pots of steam-sterilized soil containing healthy plants grown from cane cuttings. The incubation period averaged 12½ days.

The isolate of the fungus from *Dieffenbachia picta* failed to infect certain ornamentals tested in a comparable environment.

The minimum temperature for mycelial growth was 13° C., the optimum 28°, and the maximum 31°.

The disease is favored by high air temperatures, high humidity, poor soil drainage, excessive irrigation, and crowding of the potted plants in the greenhouse.

The disease can be avoided by rooting cane cuttings, previously dusted with Fermate, Phygon, or Spergon, in small pots of steam-sterilized sand. Subsequently, in shifting the rooted cuttings to larger pots, steam-sterilized soil should be used. Care in watering the plants and good drainage are essential.

CALIFORNIA AGRICULTURAL EXPERIMENT STATION,

    BERKELEY, CALIFORNIA,

        AND

MISSOURI AGRICULTURAL EXPERIMENT STATION,

    COLUMBIA, MISSOURI.

LITERATURE CITED

1. ASHBY, S. F. Strains and taxonomy of *Phytophthora palmivora* Butl. (*P. Faberi* Maubl.). Trans. Brit. Mycol. Soc. 14: 18–38. 1929.
2. BUISMAN, CHRISTINE J. Root rots caused by Phycomycetes. Typ. Hollandia-Drukkerij, Baarn, 51 pp. 1927.
3. BUTLER, E. J. An account of the genus Pythium and some Chytridiaceae. Mem. Dept. Agr., India, Bot. Ser. 1, 5: 1–160. 1907.
4. RACIBORSKI, M. Parasitische Algen und Pilze Javas. 1: 9–10. 1900.
5. TOMPKINS, C. M., and M. W. GARDNER. Relation of temperature to infection of bean and cowpea seedlings by *Rhizoctonia bataticola*. Hilgardia 9: 210–230. 1935.
6. —————, and C. M. TUCKER. Leaf blight of pink calla caused by *Phytophthora erythroseptica*. Phytopath. 37: 382–389. 1947.
7. TUCKER, C. M. Taxonomy of the genus Phytophthora deBary. Mo. Agr. Exp. Sta. Res. Bull. 153. 1931.

NITROGEN, POTASSIUM, AND CALCIUM IN RELATION TO FUSARIUM WILT OF MUSKMELON[1]

DAVID L. STODDARD

INTRODUCTION

Fusarium wilt of muskmelon (*Cucumis melo* L.) caused by *Fusarium bulbigenum* (Cke. and Mass.) var. *niveum* Wr. f. 2 has been fully described in Minnesota by Leach and Currence (6). The disease now occurs in a number of muskmelon producing areas in the United States including Maryland where it is serious in Anne Arundel County.

The relation of nutrition to disease resistance has been the subject of extensive study, and there have been numerous instances in the literature in which plant diseases have been partially controlled by the modification of fertilizer practices. A comprehensive review of this subject has been written by Wingard (17). Of particular interest are those papers pertaing to the relation of nutrition to the control of diseases caused by species of *Fusarium*.

Fisher (4), in his study of Fusarium wilt of tomato, found that a heavy application of lime plus low nitrogen reduced the number of plants invaded by the pathogen. Cook (2), working with the same disease, obtained similar results when a low nitrogen nutrient was supplied. Thomas and Mack (13), using the foliar diagnosis technique, analyzed tomato plants grown on wilt-infested soil. Sampling before symptoms of the disease appeared, they found that those plants which remained healthy contained more potassium than did those plants which subsequently became diseased. They found, also, that plants which became diseased had a low calcium content.

Sherwood (11) observed that the highest percentage of tomato wilt always occurred in the most acid soils in his experiments. Scott (10) noted that less Fusarium wilt of tomato occurred when the plants were grown at a pH of 6.4 to 7.0 and that more wilt appeared under more acid or more alkaline conditions.

Neal (8), Smith (12), Tisdale and Dick (14), and Young and Tharp (18) have reported that the application of high potash fertilizers, particularly to soils deficient in potassium, reduced the severity of wilt in cotton caused by *Fusarium vasinfectum* Atk. The effect of various nitrogen levels alone was not significant (8, 14) although Young and Tharp (18) felt that the application of sufficient amounts of potash to balance the available nitrogen and phosphorus was highly important in controlling the cotton wilt disease.

Walker and Hooker (15), in their study of cabbage yellows caused by *Fusarium conglutinans* Woll., found that, when plants were grown in sand culture, reduction of the potassium level consistently increased the severity of the disease.

The present study was undertaken to determine whether nitrogen, potassium, and calcium nutrition influences the susceptibility of muskmelon to Fusarium wilt.

<center>MATERIALS AND METHODS</center>

Inoculum and Inoculation Technique. The highly pathogenic culture of *Fusarium bulbigenum* var. *niveum* f. 2 which was used throughout the greenhouse and laboratory experiments was the third successive single spore isolate from a culture isolated from a wilted muskmelon plant. In the field experiment, an area was selected which was naturally infested with the pathogen.

Inoculum was prepared by growing the fungus on liquid Leonian's medium[2] in Erlenmeyer flasks. When the fungus mat had covered the surface of the medium (approximately 15 days at 27° C.), the cultures were filtered, and the mat was ground for one minute with tap water in a Waring Blendor. The suspension of spores and mycelial fragments was used without further treatment. Plants were inoculated by making a dibble hole in the sand adjacent to the tap root and pouring 15 ml. of inoculum on the roots thus exposed.

Culture of Plants in Greenhouse. All greenhouse experiments were planned as randomized block designs with at least four replications in each block. Seeds of the variety Bender's Surprise treated with tetra-chloro parabenzo-quinone (Spergon) were sown directly into 2-gallon crocks of steam-sterilized, quartz sand. In early experiments fine sand (predominately of 40-mesh grade) was used. When it was shown (9) that a coarse sand (predominately of 20-mesh grade) was superior for muskmelon culture, this grade was used in all subsequent experiments. The application of nutrient solutions was begun after the first true leaves appeared.

Nutrient solutions in tap water were prepared daily from molar stock solutions of the following salts: $Ca(NO_3)_2 \cdot 4H_2O$, KH_2PO_4, $MgSO_4 \cdot 7H_2O$, $CaCl_2O$, KCl, KNO_3, $Mg(NO_3)_2 \cdot 6H_2O$, $NaNO_3$. Throughout the study the following elements were supplied at constant rates as follows: calcium at 240 p.p.m., magnesium at 48 p.p.m., and phosphate at 190 p.p.m. The solutions were supplemented by the addition of measured small amounts of manganese, zinc, copper, boron, and iron. Nutrient solutions were applied to the crocks at the rate of 500 ml. once a day. Crocks were thoroughly flushed with tap water once a week.

Culture of Plants in the Field. The field experiment was conducted on a farm in Anne Arundel County. Three years before its use in this experiment, the field used was planted with muskmelons and, according to the

---

[2] Leonian's medium: potassium monobasic phosphate 1.20 gm., magnesium sulphate 0.60 gm., peptone 0.60 gm., maltose 6.25 gm., malt extract 6.25 gm., and water 1000 ml.

owner, the crop had been a total loss because of Fusarium wilt.   After the crop failure, the field lay fallow for one year and then was planted with vetch.   This cover crop was turned under in March, and in mid-June the land was prepared for muskmelons at which time soil samples were taken. Analysis[3] showed the soil to be a sandy loam with a pH value of 5.1 and containing 0.4 per cent organic matter.   Its general fertility level was low.

The design of this experiment was that of a split block with lime vs. no lime as the whole plot.   Whole plots were replicated 5 times.   Hydrated lime was applied broadcast prior to the final harrowing at the rate of 1500 lb. per acre.   This amount was calculated to raise the soil pH to 6.0.   Fertilizer formulas were applied in the bottom of the rows at the rate of 800 lb. per acre.   These formulas were 6–6–5 (normally used for muskmelons by the majority of farmers in the area), 4–8–8, 4–8–12, 4–8–16, and 4–8–20.

TABLE 1.—*System used for rating muskmelon plants infected with Fusarium wilt*

Rating	Description
0–4	Healthy or with slight to moderate discoloration of vascular system at soil line.
5–9	One branch root necrotic and with reddish-brown discoloration extending as an external streak up the tap root. Several drops of reddish-brown exudate on stem at first or second internode. Tip leaves of one runner slightly yellowed and cupped or runner nearly dead. Rest of plant normal.
10–14	One branch root necrotic and with reddish-brown discoloration extending as an external streak up the tap root. Brown discolored streak on approximately half of main stem. Streak may also extend part way along branch stem. Leaves more or less yellowed and cupped on stems with a streak. One or more branch stems normal.
15–19	One or more branch roots necrotic and with reddish-brown streak extending externally up the tap root. Brown streak on main stem and on all branch stems except one. Leaves more or less yellowed and cupped on stems with streak. One or more branches dead.
20	Entire plant dead.

Muskmelon plants, Clark variety, were propagated from seed in No. 2 tin cans filled with a compost-sand mixture in a cold frame.   When two true leaves had appeared they were transplanted to the field.   All plants received the same fertilizer in the cans that they later received in the field. Fifty-four days after transplanting, all plants were examined and rated for Fusarium wilt.

Disease Criteria.   In the first greenhouse experiments the plants were rated as either dead or healthy.   In subsequent experiments plants were rated according to a system developed for the field experiment.   A description of the classes in this system appears in table 1 and diagrams of plants in some of the classes are shown in figure 1.   Within each classification, consideration was given to the total amount of the plant affected including the extent to which fruit-bearing was prevented.   Thus, a diseased plant which matured a marketable fruit on an apparently healthy runner received

a slightly lower rating than a similarly diseased plant which matured no fruit. In the last greenhouse experiment the plants were cut at the ground line, and freehand sections were made one inch below the cotyledonary node. Disease ratings were made on the basis of the percentage of the cross section of the stem invaded by the fungus hyphae.

Nutrition Experiments on the Pathogen. Spore suspensions of the pathogen were transferred to flasks containing 25 ml. of autoclaved nutrient
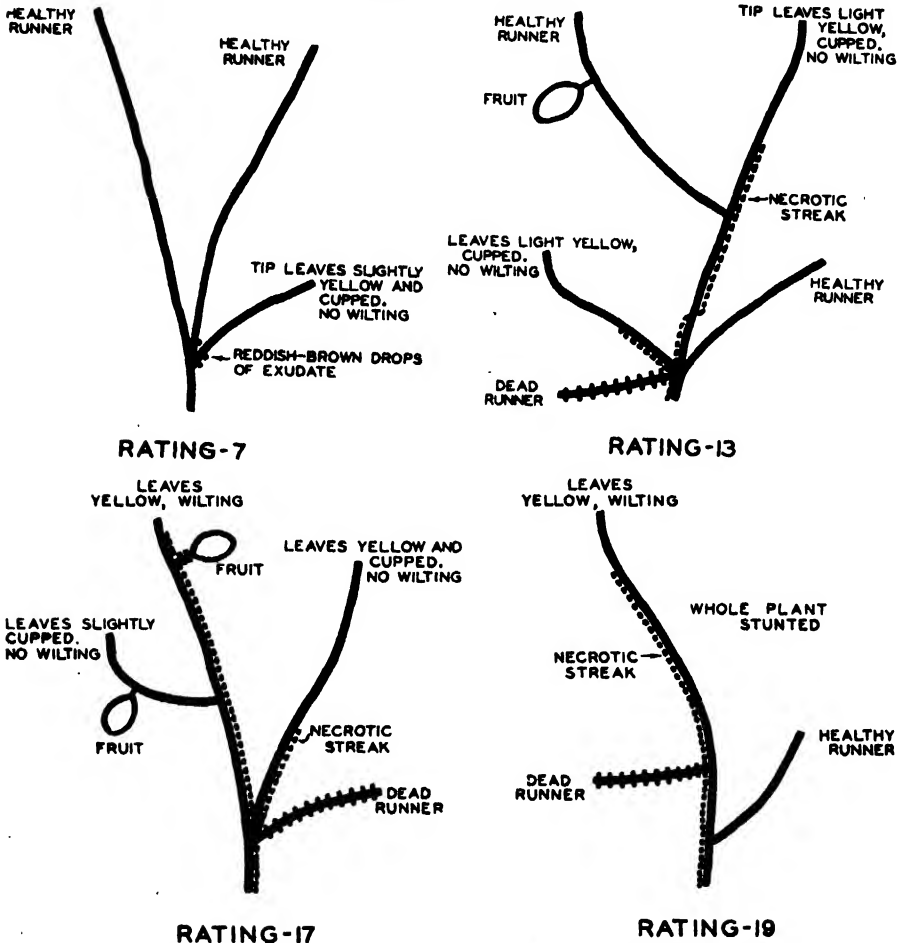
FIG. 1. Diagrams of four muskmelon plants infected with Fusarium wilt and given disease ratings as indicated.

solutions identical with those supplied to the muskmelon plants. After ten days' growth at 27° C., the mats were filtered off, oven dried, and weighed. In a second experiment the same procedure was followed except that nutrient solutions were divided into three pH series; pH 3.3, pH 6.0, and pH 8.0. The three series were buffered and adjusted to their respective pH levels. Representative flasks were titrated every three days and the pH of all flasks adjusted.

RESULTS

Greenhouse Experiments. Preliminary tests were run to determine the nutrient balance necessary for optimal growth of muskmelon plants in quartz sand and to ascertain the differences in nitrate content of main stems of plants grown on varying levels of nitrogen and potassium. The results showed that no significant increase in growth occurred after a level of 447 p.p.m. of nitrate was reached and that variations in the potassium level had no significant effect on total growth, although plants receiving 117 and 156 p.p.m. of potassium made more growth than did those receiving 78 and 195 p.p.m. The nitrate content of the stems, as analyzed by the rapid method of Carolus (1), had a high correlation with that supplied in the nutrient solution. At the lowest level of potassium (78 p.p.m.) there was significantly more nitrate than at any of the higher levels (117, 156, and 195 p.p.m.). At 447 p.p.m. of nitrate and above, regardless of the level of po-

TABLE 2.—*Correlations between the amount of nitrate and the amount of potassium in the nutrient solution and the number of muskmelon plants killed by Fusarium wilt*

P.p.m. nitrate	Plants killed[a]	P.p.m. potassium	Plants killed[b]
100	10	78	28
275	12	117	24
447	17	156	19
620	25	195	19
744	26		
$r = 0.977$**		$r = -0.947$*	

** Significant at the 1 per cent point.
* Significant at the 5 per cent point.
[a] Total of 32 plants in each class.
[b] Total of 40 plants in each class.

tassium, the plants had a healthy green appearance. At concentrations of nitrate below 447 p.p.m., plants were a light green and resembled plants with symptoms of nitrogen deficiency.

Since, in the first inoculation experiment, results were recorded simply as plants killed by the pathogen, correlations on the relation between the number of plants killed and the amount of nitrate and the amount of potassium supplied were calculated. The results (Table 2) show that there was a highly significant correlation between mortality and the amount of nitrate supplied. The correlation between mortality and the potassium supply was significant at the 5 per cent point.

In the next experiment the plants were removed from the sand thirty days after inoculation and rated according to the system developed for the field experiment (Fig. 1 and Table 1). The results (Table 3) show a highly significant difference between the 100 p.p.m. and the 447, 744, and 992 p.p.m. nitrate levels but no significant difference between potassium levels or for the interaction between nitrate and potassium.

Fusarium was reisolated from every plant in the experiment irrespective of disease rating. The 160 isolates obtained were classified into eight

TABLE 3.—*Effect of inoculation with Fusarium wilt organism on muskmelon plants grown in quartz sand under various levels of nitrate and potassium*

P.p.m. nitrate	P.p.m. potassium				Mean disease rating per plant
	78	195	351	507	
100	0.7[a]	6.0	8.0	8.5	5.8
447	11.5	9.7	11.8	12.7	11.4
744	13.6	13.2	7.7	12.2	11.7
992	11.2	10.0	8.0	14.0	10.8
Mean disease rating per plant	9.3	9.7	8.9	11.9	

L.S.D. at 1 per cent point between means—4.2
L.S.D. at 5 per cent point between means—2.2

[a] Basic figures represent average rating for 10 plants.

different groups according to gross morphological differences and a representative from each was tested for pathogenicity on young muskmelon seedlings. All groups were pathogenic except one consisting of two isolates.

Since it has been shown in the previous experiment that even apparently healthy plants were invaded by the Fusarium wilt fungus, an experiment was set up to determine the extent to which plants would be invaded when grown under different nutritional levels. The data (Table 4) show a highly significant difference between the 100 p.p.m. level of nitrate and the 447 and 744 p.p.m. levels. There was no significance among any of the potassium levels or in the nitrate-potassium interaction.

Field Experiment. Preparatory to this experiment, all of Anne Arundel County was surveyed to determine the extent of the disease and the cultural procedures commonly used in the production of muskmelons. The fertilizer ratio generally used by the growers was 6–6–5. On the majority of farms, lime was seldom used and most of the soil tested was in the pH range of 4.5 to 5.5. In one field visited, the apparent result of soil acidity was striking. This field was divided into three definite sections: (1) all plants healthy, soil pH of 5.5, (2) 95 per cent of the plants dead from Fusarium

TABLE 4.—*Cumulative percentage invasion by the Fusarium wilt fungus of the hypocotyl of plants grown on various levels of nitrate and potassium*

P.p.m. nitrate	P.p.m. potassium			Total percentage[a] invasion
	78	156	273	
100	23	44	116	183
447	139	843	725	1707
744	731	723	858	2312
Total percentage[a] invasion	893	1610	1699	

L.S.D. at 1 per cent point between totals—1468
L.S.D. at 5 per cent point between totals—1074

[a] Represents total of 54 plants.

wilt, soil pH 4.1, and (3) 50 per cent of the plants dead or severely affected by Fusarium wilt, soil pH 4.8. In many other fields visited, calcium deficiency symptoms were noted. Because of these observations, it was decided to include in the field experiment lime plots in which the pH would be raised to 6.0, a level consistent with that recommended for muskmelon culture (5, 16).

The data obtained (Table 5) showed highly significant differences between the limed and unlimed plots. There were no interaction effects between lime and any of the fertilizer ratios used. The difference beween the check fertilizer, 6–6–5, and the other ratios was highly significant: there was no significance within the 4 per cent nitrogen series except that the 4–8–20 ratio was significantly better, at the 5 per cent point, than the 4–8–8 and 4–8–16 ratios.

TABLE 5.—*Amount of Fusarium wilt in muskmelon plants grown in wilt-infested soil modified by lime and various fertilizer ratios*

| | Fertilizer ratio | | | | | Mean disease rating per plot |
	6–6–5	4–8–8	4–8–12	4–8–16	4–8–20	
Lime	5.9[a]	4.2	3.4	3.4	3.0	3.95
No lime	8.8	5.2	4.9	5.9	3.3	5.63
Mean disease rating per plot	7.32	4.69	4.15	4.65	3.15	

L.S.D. at 1 per cent point between means for lime—0.83
L.S.D. at 1 per cent point between means for fertilizers—1.65
L.S.D. at 5 per cent point between means for fertilizers—1.25

[a] Basic figures in table are averages of 5 plots.

Effect of Nutrient Solutions on Growth of the Pathogen in Vitro. In a preliminary experiment the pH of the nutrient solutions was not controlled. The results showed a high correlation between the total growth of the pathogen and the amount of nitrogen in the medium. Levels of potassium of 351 and 429 p.p.m. produced significantly less growth than did the 273 p.p.m. level.

In the experiment where the pH of the nutrient solutions was controlled the fungus tended to raise the pH from pH 3.3 and lower the pH from pH 6.0 and 8.0. The total weights in milligrams of the *Fusarium* were 1244 mg. at pH 3.3, 274 mg. at pH 6.0, and 585 mg. at pH 8.0. The L.S.D. at the 1 per cent point for these weights was calculated to be 124.4 mg. Thus the differences among all three pH levels was highly significant. The complete analysis of variance for this experiment appears in table 6. It should be noted that the variance for pH levels is very large. In order to evaluate more fully the effects of pH on the growth of the fungus, the figures within each pH series were analyzed separately. The data are presented in table 7. At pH 3.3 there was a highly significant increase in growth of the fungus with each increase in nitrate level. At pH 6.0 no significant increase in

TABLE 6.—*Analysis of variance of experiment in which the Fusarium wilt organism was grown for ten days under various levels of nitrate and potassium within each of three pH series*

Source of variation	Degrees of freedom	Variance	"F" value
Total	107		
Nitrate	2	1714.75	52.940*
Potassium	2	5.50	0.190
pH	2	6814.35	210.384**
$NO_3 \times K$	4	52.62	1.613
$NO_3 \times pH$	4	164.02	5.064**
$K \times pH$	4	129.07	3.985**
$NO_3 \times K \times pH$	8	241.24	7.448**
Error	81	32.39	

** Significant at the 1 per cent point.

growth occurred until the highest level of nitrate was attained, whereas at pH 8.0 there were no significant differences in total growth between any of the nitrate levels. When pH was held at 3.3, solutions containing 78 p.p.m. of potassium produced significantly (at 5 per cent point) more growth than did solutions containing 195 p.p.m. of potassium. When the fungus was grown at pH 6.0 and 8.0, potassium levels had no effect on total growth.

TABLE 7.—*Growth in milligrams (dry weight) of Fusarium bulbigenum var. niveum f. 2 in nutrient solutions containing three levels of nitrate and potassium and held at three pH levels for ten days*

pH	P.p.m. nitrate	P.p.m. potassium			Mean
		78	156	195	
	100	24.8[a]	25.8	19.5	23.3
3.3	273	40.0	39.8	30.5	36.7
	447	46.3	41.8	42.8	43.6
	Mean	37.0	35.7	30.9	

L.S.D. at 1 per cent point between means—6.8
L.S.D. at 5 per cent point between means—5.0

pH	P.p.m. nitrate	78	156	195	Mean
	100	−1.3[a]	2.3	4.3	1.8
6.0	273	2.5	2.8	9.3	4.8
	447	14.0	23.5	11.3	16.3
	Mean	5.1	9.5	8.3	

L.S.D. at 1 per cent point between means—9.2
L.S.D. at 5 per cent point between means—6.8

pH	P.p.m. nitrate	78	156	195	Mean
	100	14.3[a]	6.5	19.8	13.5
8.0	273	18.5	15.3	18.3	17.3
	447	19.5	18.3	16.0	17.9
	Mean	17.4	13.3	18.0	

Differences between means not significant.

[a] Basic figures represent average weights of 4 replications.

DISCUSSION

Throughout the course of this study, a relationship between nitrogen nutrition and the behavior of *Fusarium bulbigenum* var. *niveum* f. 2 has been observed. In the greenhouse inoculation experiments an increase of nitrogen in the nutrient solution resulted in a significantly greater invasion of the plant by the fungus and a larger number of the plants killed. There is little evidence that potassium plays an important part in affecting susceptibility when nitrogen and potassium are balanced and in amounts sufficient for normal growth of the muskmelon plant. In the field experiment the highly significant reduction in the amount of disease occurred between the 6-6-5 and the 4-8-8, 4-8-12, 4-8-16, and 4-8-20 fertilizer ratios. Within the 4 per cent nitrogen ratios there were no highly significant differences. It is possible that these results can be attributed more to the reduction of the percentage of nitrogen rather than to an increased percentage of potassium.

The nutrition studies of the *Fusarium, in vitro*, indicate that nitrogen is the important element governing the growth of the fungus. Cox (3), in his work on the host-parasite relationship in muskmelon wilt, found that in the early stages of the disease the fungus enters the roots and primarily inhabits the xylem although other tissues are invaded later. It appears, then, that the *Fusarium* must depend largely, at least in the early stages of infection, on elements in the xylem stream for its nutrition. It is possible that an abundance of soluble nitrogen in the xylem could influence the extent of invasion. The possibility that an abundance of nitrogen makes host cells more susceptible to invasion by the parasite cannot be overlooked.

The effects of pH on the amount of disease present in the field were similar to those reported by Sherwood (11) and Scott (12) who worked with Fusarium wilt of tomato. The field results with lime also parallel the data obtained in the laboratory where the fungus, *in vitro*, made the poorest growth when held at a pH of 6.0. Since there was no significant interaction between lime and any of the fertilizer ratios, it is possible that the lowered disease index in the limed plots resulted from inhibiting the growth of the fungus in the soil or in lowering its virulence. It is recognized, however, that muskmelons grow best when the soil reaction is slightly acid or neutral (5, 16). That muskmelons have a high calcium requirement is also well known (7). Some of the beneficial effects of lime, therefore, may have come from providing the plants with soil conditions that were more nearly optimum.

SUMMARY

The observations obtained from greenhouse and field experiments indicate that high levels of nitrogen result in a significantly greater invasion of muskmelon plants by *Fusarium bulbigenum* var. *niveum* f. 2 and a larger number of plants killed. There was little evidence that potassium

plays an important part in affecting susceptibility when nitrogen and potassium are balanced and in amounts sufficient for normal growth of the muskmelon plant. *In vitro*, the pathogen made more total growth as the nitrogen content of the medium was increased; however, little correlation was observed between total growth and potassium supply.

The addition of lime to the soil in an amount sufficient to raise the soil pH to 6.0 significantly reduced the amount of disease in the field plots. Part of the reduction may be attributed to the change in pH since, *in vitro*, the growth of the pathogen was drastically inhibited when the pH of the medium was held at 6.0.

Botany Department,
University of Maryland,
College Park, Maryland.

LITERATURE CITED

1. Carolus, R. L. The use of rapid chemical plant nutrient tests in fertilizer deficiency diseases and vegetable crop research. Va. Truck Exp. Sta. Bul. 98. 1938.
2. Cook, William S. Relation of nutrition of the tomato to disposition to infectivity and virulence of *Fusarium lycopersici*. Bot. Gaz. 98: 647–669. 1937.
3. Cox, Carroll E. The host-parasite relationship in susceptible cantaloups, resistant cantaloups and cucumbers inoculated with *Fusarium bulbigenum* (Cke. and Mass.) var. *niveum* Wr. f. 2. Ph.D. Thesis (unpublished), University of Maryland. 1943.
4. Fisher, Paul Lewis. Physiological studies on the pathogenicity of *Fusarium lycopersici* for the tomato plant. Md. Ag. Exp. Sta. Bul. 374. 1935.
5. Jones, Henry A. and Joseph T. Rosa. Truck crop plants. 538 pp. New York: McGraw-Hill Company, Inc. 1928.
6. Leach, J. G., and T. M. Currence. Fusarium wilt of muskmelons in Minnesota. Minn. Agr. Exp. Sta. Tech. Bul. 129. 1938.
7. Miller, Edwin C. Plant Physiology. 1201 pp. New York: McGraw-Hill Company, Inc. 1938.
8. Neal, David C. Cotton wilt: a pathological and physiological investigation. Ann. Mo. Bot. Gard. 14: 359–424. 1927.
9. Pierce, E. C., and D. L. Stoddard. Some effects of sand and nutrient supply on a physiological leaf spot of cantaloupe. Phytopath. 33: 162–164. 1943.
10. Scott, I. T. The influence of hydrogen-ion concentration on the growth of *Fusarium lycopersici* and on tomato wilt. Mo. Agr. Exp. Sta. Res. Bul. 64. 1924.
11. Sherwood, E. C. Hydrogen-ion concentration as related to the Fusarium wilt of tomato seedlings. Amer. Jour. Bot. 10: 537–553. 1923.
12. Smith, Albert L. A regional study of the relationship of potash treatments to the development of cotton wilt under widely varying conditions of soil and environment. Proc. Soil Sci. Soc. Amer. 4: 332. 1939.
13. Thomas, Walter, and Warren B. Mack. A foliar diagnosis study of the nutrition of greenhouse tomatoes in relation to the incidence of a disease. Penn. Agr. Exp. Sta. Bul. 405. 1941.
14. Tisdale, H. B., and J. B. Dick. The development of wilt in a wilt-resistant and a wilt-susceptible variety of cotton as affected by N-P-K ratio in fertilizer. Proc. Soil Sci. Soc. Amer. 4: 333–334. 1939.
15. Walker, J. C., and W. J. Hooker. The relation of host nutrition to the development of cabbage yellows. (Abstr.) Phytopath. 33: 15. 1943.
16. Watts, Ralph L., and Gilbert Searle Watts. The vegetable growing business. 520 pp. New York: Orange Judd Publishing Company. 1940.
17. Wingard, S. A. The nature of disease resistance in plants. Bot. Rev. 7: 59–109. 1941.
18. Young, V. H., and W. H. Tharp. Relation of fertilizer balance to potash hunger and the Fusarium wilt of cotton. Ark. Agr. Exp. Sta. Bul. 410. 1941.

A PHYSODERMA DISEASE OF QUACK GRASS

M. J. THIRUMALACHAR AND J. G. DICKSON

While searching for the stripe smut *Ustilago striiformis* (West) Niessl on various grasses in the fields about Madison, Wisconsin, an apparently new disease was found on *Agropyron repens* (L.) Beauv. A cursory study revealed that the disease was incited by a species of *Physoderma,* new for Wisconsin and perhaps for the United States.

The symptoms of the disease are distinctive. Internodal elongation of the culms is reduced and the leaf blades develop erectly. The young leaves are light green and the older leaves have small pale yellow striae that gradually change to rusty brown stripes as the numerous striae coalesce (Fig. 1, B). In external appearance the symptoms simulate those of downy mildew on the grasses. The mode of development suggests a systemic type of infection although the resting sporangia are produced in the leaf blades and sheaths. Basal rotting of the culm and wilting and drying out of the leaves occurs in the case of the more severe infection (Fig. 1, A). No infection of the root cortical tissues has been evident in the field material.

Sections through the infected leaves show numerous resting sporangia of a species of *Physoderma.* In the initial stages of fungus development in the young tissues, the delicate rhizomycelium is evident in the host cells. The sporangia form, filling the parenchymatous cells, and resemble in essential features those of *Physoderma zeae-maydis* Shaw on corn. Mature sporangia are reddish-brown, thick-walled, smooth, 20–40 μ in diameter with granular cell contents. The spores are slightly flattened on one side indicating the position of the operculum (Fig. 2).

Sporangia scraped out of mature sori were placed in small quantities of water on slides and inclosed in moist chambers at 24°–30° C. Germination of a few sporangia that were observed was similar to that described by Tisdale (5) for *Physoderma zeae-maydis.* The operculum is pushed aside by the protrusion of the endospore and the contents of the sporangium form numerous zoospores. Further observations are being made on the behavior of the zoospores and the mode of infection.

The fungus under study closely resembles *Physoderma zeae-maydis* on corn, but differs slightly in the type of symptoms produced as well as in the measurement of the sporangia. In *P. zeae-maydis,* the infection tends to be localized, whereas in the *Physoderma* under study, the infection is probably systemic. Furthermore, in the former the mature sori are erumpent, whereas in the latter, the sori are nonerumpent and the sporangia are released only by the decay of the leaf tissue. Sporangia of *P. zeae-maydis* measure 18–24 × 20–30 μ in comparison with 20–40 × 20–34 μ in the *Physoderma* on quack grass.

FIG. 1. Culms of *Agropyron repens* infected by *Physoderma*. A. Plants with stripes on the leaves, stunted growth, and rotting at the base of the culms. × ¼ nat. size. B. Enlarged leaf blade showing the numerous striae coalescing to form brown stripes.
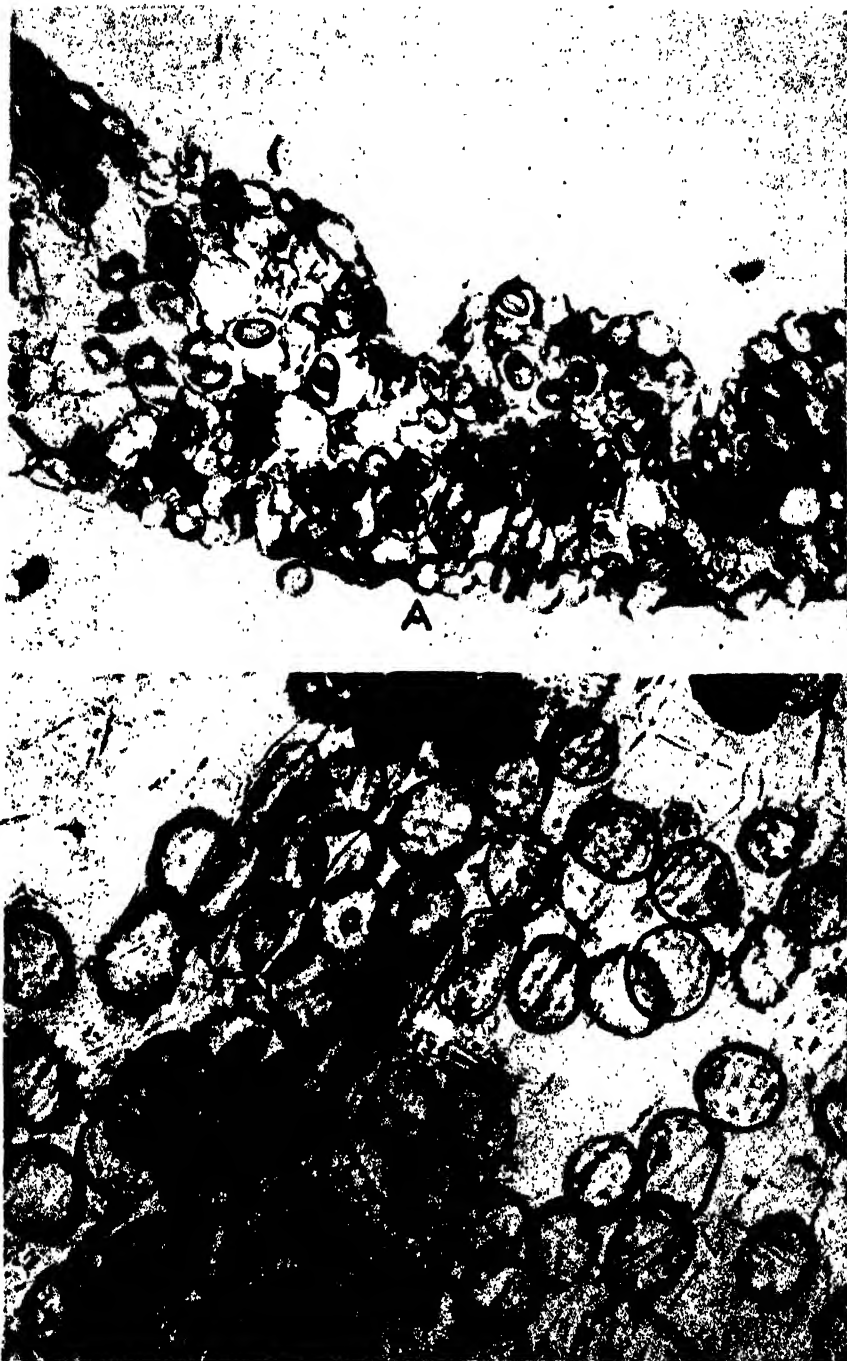
Fig. 2.   Resting sporangia of the *Physoderma* on *Agropyron repens*.   A. Transection through the diseased leaf showing the sporangia in the mesophyll cells.   About 80 ×. B. Typical masses of the sporangia.   About 300 ×.

A similar disease has been described in Europe on several economic grasses. Sampson and Western (3, p. 33) described a yellowing and damping-off of grass seedlings especially *Agrostis spp.* when in the first or second leaf stage as probably due to *Cladochytrium caespitis* Griff. and Maubl. This species is similar to *Physoderma* as discussed by Cook (1) who combined the two genera; Sparrow (4) and others, however, retain the two genera. *Physoderma agrostidis* Lagerh. on *Agrostis gigantea* Roth and *P. graminis* (Büsgen) Fischer on *Dactylis glomerata* L., *Agropyron* (*Triticum*) *repens* (L.) Beauv., *Alopecurus pratensis* L., and *Phleum pratense* L. have been described in Europe (2). The species found near Madison, Wisconsin closely resembles *P. graminis* in morphology; however, sporangia have not been found in the root cortex as described for this species. The disease was not found on the other three grasses although they were growing in close proximity to the infected quack grass. In so far as the authors can determine this is the first report of this fungus in North America.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

LITERATURE CITED

1. COOK, W. R. I. Some observations on the genus Cladochytrium with special reference to *C. caespitis* Griffin and Maublanc. Ann. Bot. **48**: 177–185. 1934.
2. MINDEN, VON M. Myxomycetes, I Reihe: Chytridiineae. Kriptogamenflora der Mark Brandenburg **5**: 209–422. 1915.
3. SAMPSON, K., and J. H. WESTERN. Diseases of British grasses and herbage legumes. 85 pp. University Press, Cambridge, England. 1941.
4. SPARROW, F. K. Aquatic Phycomycetes exclusive of the Saprolegniaceae and Pythium. 785 pp. Univ. of Mich. Press. 1943.
5. TISDALE, W. H. Physoderma disease of corn. Jour. Agr. Res. [U.S.] **16**: 137–154. 1919.

PHYSIOLOGIC RACES OF USTILAGO TRITICI IN THE EASTERN SOFT WHEAT REGION OF THE UNITED STATES[1]

WAYNE M. BEVER[2]

INTRODUCTION

The first published description of physiologic races of *Ustilago tritici* (Pers.) Rostr. based on pathogenicity tests was by Piekenbrock (9) in 1927. Since that time other workers (3, 4, 5, 6, 7, 8, 11) have described a number of races of this fungus. In 1930 Grevel (4) working in Germany reported four races of loose smut of wheat. Radulescu (11) found no indication of physiologic specialization when he tested seven collections of loose smut from Romania on five standard winter wheat varieties. He did, however, find three races when he used twelve varieties of spring wheat as host differentials in testing eight collections of loose smut. In 1932 Hanna and Popp (6) working in Canada described two races of loose smut based on the reactions of common and durum wheats. Again in 1937 Hanna (5) described two additional races determined by tests on nine varieties of spring wheat, two durum varieties, one durum × spring wheat hybrid, and one emmer. In 1936 and in 1942 Moore (7, 8) reported five physiologic races that he had distinguished by reactions of certain bread and durum wheats, but did not name the varieties nor their reactions. These races were derived from twelve smut collections from Minnesota, North Dakota, Texas, and Mexico. Caldwell and Compton (3) reported host specialization among seven wheat loose smut collections from Illinois, Indiana, and Ohio. Working independently in Texas, Kansas, and Illinois on a study of wheat varietal reaction to loose smut, Atkins, Hansing, and Bever (1) found that the same variety might be susceptible at one location and not at another, which indicated that different physiologic races of the fungus were occurring in the different areas.

In 1940, as a part of the coordinated eastern soft winter wheat program, the writer started an intensive study on the problem of physiologic specialization in *Ustilago tritici*. This study involved pathogenicity tests of smut collections from all the wheat growing regions of the United States with special emphasis on smut collections from the eastern soft wheat region. The results here reported cover primarily smut from this latter region.

MATERIALS AND METHODS

The work was done at the Illinois Agricultural Experiment Station, Urbana, Illinois. All of the inoculations were made in the greenhouse.

The following winter wheat varieties were used as differentials: Wabash (C.I.[3] 11384), American Banner (C.I. 6943), Purdue No. 1 (C.I. 11380), Hussar (C.I. 4843), Early Premium (C.I. 11858), Nabob (C.I. 8869), Forward (C.I. 6691), Trumbull (C.I. 5657), Kanred-Gipsy (C.I. 11382), Leap (C.I. 4823), Kawvale (C.I. 8180), and Prairie (C.I. 12371). The results with Kawvale and Prairie are not included here because their reactions did not differentiate any of the smut collections thus far studied. Kawvale was resistant to all of them. Prairie was susceptible to most of them. The varieties were selected on the basis of physiologic race investigations by Caldwell and Compton and because of their possibilities as parent material in breeding for resistance to loose smut.

For the most part, the inoculum was collected prior to 1940 by B. B. Bayles, Division of Cereal Crops and Diseases, and each collection inoculated separately into the spring wheat variety Kota. The infected seed was given to the writer and the original inoculum employed in this study was taken from smutted plants grown from these various lots of infected Kota seed.

The smut inoculum for the first year's test was taken from the variety Kota. For the second year's test it was taken from two of the differential varieties and mixed, except that race 1 was taken from Wabash which was the only host differential infected. For the third and fourth year's test the inoculum was taken from the same variety each year.

In all, 52 smut collections from Texas, Missouri, Michigan and eastward have been classified into races on the basis of the reactions of the differential varieties grown in the field from infected seed produced by plants inoculated in the greenhouse.

The seed of the differential varieties to be inoculated was treated by the modified hot-water treatment to insure smut free plants. After the seed had dried for three or four days, it was treated with an excess of Semesan Jr. (one per cent ethyl mercury phosphate) as a precaution against contaminants during the vernalization process, which was as follows: From 300 to 400 kernels of the treated seed of each variety were put in a separate 155-mm. Petri dish with 20 cc. distilled water added and left at room temperature for 24 hours to start germination. The Petri dishes were then placed in a refrigerator at 2° to 4° C. and left 60 days, water being added as necessary. This period of vernalization was found optimum for most of the varieties. At the end of the 60 days the coleoptiles of most varieties were one-half to two and a half inches long and, in some varieties, the first leaf had pushed out of the coleoptile about one inch. The young seedlings were then planted in soil in 2-gallon glazed jars, 15–17 plants per jar in the greenhouse.

[3] C.I. refers to accession number of the Division of Cereal Crops and Diseases.

The soil in each jar was fertilized with 150 cc. nutrient solution[4] just before planting and again at each 10-day interval until the plants were in the early boot stage.

Two crops a year were grown in the greenhouse. For the fall crop, the the seed was vernalized during July and August and planted in early September; and the plants matured early in January. The spring crop was handled about four and a half months later.

The greenhouse temperature ranged from 50° to 95° F. However, as the season progressed a temperature of 60° to 70° F. was maintained until the plants were in the soft-dough stage when it was raised to 75° to 80° F. until the plants were mature.

During the short days of late fall and winter, daylight was supplemented by three 750-watt electric bulbs supplying 150 to 180 candle power, for each 2.5 × 17 foot bench, daily to March 15. By seeding in early September very little floral sterility was encountered.

At anthesis, which was approximately 60 days after the seedlings were planted in the jars, four heads of each of the differential varieties were inoculated with a malt-extract[5] suspension of chlamydospores of each smut collection. A spore dilution of 0.03 gm. spores per 100 cc. of malt extract was used. Other dilutions were tried but were less satisfactory. The method of inoculation described by Poehlman (10) was used except that a regular 20-cc. hypodermic syringe was employed instead of the rubber bulb, and malt extract was used instead of dextrose. The use of the hypodermic syringe and needle has several advantages, namely: a small amount of inoculum is required, adequate inoculum is assured for each floret, and there is little or no head mortality.

After the inoculated heads had matured, they were harvested and threshed individually. The seed from the 4 heads inoculated with the same smut collection was thoroughly mixed and divided into four groups and packeted. At fall seeding time each of the four lots of inoculated seed was sown in a separate hill in rows as described by Bonnett and Bever (2), 15 to 30 seeds in each hill and the hills 18 inches apart in rows two feet apart.

The infected seed of the winter-tender variety Leap was sown at the Plant Industry Station, Beltsville, Maryland, each year because the danger of winterkilling there is less than at Urbana, Illinois.

[4] The nutrient solution was made by mixing 125 cc. from each of the following stock solutions A, B, and C, with 18,900 cc. tap water.

    Stock solution A
        Sodium nitrate ($NaNO_3$) .... 1012.05 gm.
        Distilled water .... 7500.00 cc.
    Stock solution B
        Sodium phosphate, monobasic, ($NaH_2PO_4 \cdot H_2O$) .... 100.12 gm.
        Distilled water .... 7500.00 cc.
    Stock solution C
        Potassium sulphate (K_2SO_4) .... 100.00 gm.
        Distilled water .... 7500.00 cc.

[5] One per cent malt extract in tap water.

The percentage of smut was based on counts of the total number of heads in the four hills. The number of heads per hill ranged from 30 to 110, and thus there were from 120 to 440 heads for each variety each year.

After the first year the smut inoculum was secured from the same variety each year and stored in a refrigerator at 40° F. until used the following winter.

For clarity and simplicity, simple numbers are used to designate the races rather than pedigree numbers as employed by Hanna (5). Infection percentages are given in terms of the nearest whole number.

In the analysis of the data, two infection classes are considered. Any variety with 10 per cent smut infection or less is considered resistant. Varieties with 11 per cent or more are considered susceptible.

RESULTS

The average percentages of smutted heads of the differential varieties produced by the different races are given in table 1. The upper figure of each pair represents the average percentage infection from individual

TABLE 1.—*Average percentages of smutted heads in 10 varieties of winter wheat inoculated with 11 physiologic races of Ustilago tritici*

Physiologic race no.	Average percentage smutted heads in									
	Wabash	American Banner	Purdue No. 1	Hussar	Early Premium	Nabob	Forward	Trumbull	Kanred-Gipsy	Leap
1	38	0	0	0	0	0	0	0	0	0
	45	0	0	0	0	0	0	0	0	0
2	46	20	18	14	0	0	14	0	0	0
	50	21	12	34	0	0	7	0	0	0
3	33	20	21	19	30	10	2	3	0	0
	43	24	25	29	32	17	0	0	0	0
4	40	43	44	19	26	25	10	0	0	0
	41	56	53	16	56	24	21	0	0	0
5	40	39	29	21	49	17	17	35	2	0
	38	40	16	10	35	20	10	32	0	0
6	42	33	28	14	42	15	22	42	24	7
	43	35	22	12	29	27	10	43	23	12
7	41	31	24	27	30	18	21	38	0	9
	44	55	12	12	56	11	17	40	0	21
8	58	0	43	29	0	0	48	39	0	0
	48	0	38	18	0	0	30	42	0	0
9	72	0	22	6	0	0	0	43	0	0
	48	0	20	0	0	2	1	50	0	0
10	56	0	0	0	0	0	0	1	37	0
	35	0	0	0	0	0	0	0	49	0
11	4	42	9	56	0	0	0	0	0	0
	0	51	0	40	0	0	0	0	0	0

ᵃ Upper figure of each pair represents the average percentage infection from individual collections during three years and the lower figure gives that obtained in one year from inoculum composited from collections that had given similar results for three years previously.

collections during three years and the lower figure of each pair gives the average percentage obtained in one year from inoculum composited from collections that had given similar percentages of smut for the three years previously.

In most cases each race is distinguished from the others by the reaction of more than one variety. The difference between races on the differentiating varieties usually was 20 per cent or more. The consistency of the results indicates that the smut inoculum was free from race mixtures.

No variety was susceptible to all of the races. While Wabash was susceptible to more races than any of the other varieties tested, it was resistant to race 11.

In certain years the plants in three hills of a variety smutted with a particular smut collection showed heavy infection, while the fourth hill had

TABLE 2.—*Distinguishing characteristics and original sources of 11 physiologic races of Ustilago tritici*

| No. | Race | Original collections from— | |
	Distinguishing characteristics	Variety	State
1	Wabash susceptible; all other differentials resistant.	Wabash, Purplestraw, Fultz, Fulcaster, Posey's Bluestem, Redrock	Ga., Ill., Ind., Ky., Mich., Mo., Tex.
2	Wabash, American Banner, Purdue No. 1, Hussar susceptible; others resistant.	Purplestraw, Honor, Forward, Clarkan	Ga., N. C., N. Y.
3	Differs from race 2 in that Early Premium and Nabob are susceptible and Forward is resistant.	Purplestraw, Fulcaster, Mediterranean, Grandprize	Ga., Ky., N. C., S. C., Tex.
4	Differs from race 3 in that Forward is susceptible.	Goens	Ky.
5	Differs from race 4 in that Trumbull is susceptible.	Purplestraw, Fultz	S. C., Ga., Tex.
6	Differs from race 5 in that Kanred-Gipsy is susceptible.	Purplestraw, Illinois No. 2, Early Premium	Ga., Md., N. C.
7	Differs from race 6 in that Kanred-Gipsy is resistant and from race 5 in that Leap is susceptible.	Trumbull, Fulcaster, Purplestraw	Ill., Ky., N. C., S. C., Tenn., Va.
8	Similar to race 2, but differs from it in that American Banner is resistant, Trumbull susceptible, and Forward susceptible.	Forward	N. C.
9	Similar to race 1, but differs in that Purdue No. 1 and Trumbull are susceptible.	Fultz	Tex.
10	Similar to race 1, but differs in that Kanred-Gipsy is susceptible.	Hope-Turkey, Fulcaster, Red May	Ark., Ind., Tex.
11	Similar to race 1, but differs in that Wabash is resistant and American Banner and Hussar are susceptible.	Turkey and two mixed varieties	Ill., Ohio, Tex.

TABLE 3.—*Number and distribution of races of Ustilago tritici in 52 collections from the eastern soft wheat area of United States*

State	Number of collections of races—											Total Total number of races
	1	2	3	4	5	6	7	8	9	10	11	
New York		1										1 1
Ohio										1		1 1
Indiana	1									2		3 2
Illinois	1						1				1	3 3
Michigan	1											1 1
Missouri	2											2 1
Maryland						1						1 1
Virginia							1					1 1
North Carolina		1	1			1	2	1				6 5
South Carolina			5		1		1					7 3
Georgia	1	1	2		1	2						7 5
Kentucky	2		1	1			1					5 4
Tennessee							1					1 1
Arkansas										1		1 1
Texas	3		1		1				1	5	1	12 6
Total	11	3	10	1	3	4	7	1	1	8	3	52
Percentage	21	6	19	2	6	8	13	2	2	15	6	

little or no smut. This was especially true previous to this experiment when the seed from the four inoculated heads was kept separate. Even though this did occur, the total percentage of heads of each variety smutted by each race was fairly constant from year to year.

The distinguishing characteristics of the 11 races given in table 1, the varietal source of each, and the state in which the original collections were made are given in table 2.

The 11 races were collected in 15 states as shown in table 3. Race 1 represented 21 per cent of the collections and was the most widespread of the races. It was collected in Georgia, Illinois, Indiana, Kentucky, Michigan, Missouri, and Texas. Race 3 comprised 19 per cent of the collections studied. Race 3 was collected in Georgia, Kentucky, North Carolina, South Carolina, and Texas. Races 7 and 10 comprised 13 and 15 per cent of the collections, respectively. Races 4, 8, and 9 were represented by 1 collection each, from Kentucky, North Carolina, and Texas. More races were represented in collections from Texas than in those from any other state.

DISCUSSION

From the 52 collections of *Ustilago tritici* on soft winter wheats from New York to Texas, 11 physiologic races have been identified on the basis of pathogenicity tests on 10 varieties of winter wheat. Sufficient data are not available to determine completely the distribution of each race, but from the data at hand, it appears that race 1 is the most widely distributed. It was collected 11 times on various varieties.

From the results as given in table 1, it seems that the smut cultures used in this study were genetically stable. On the other hand, from the nature of the life cycle of the fungus it might be that some of the cultures are in

a heterozygous condition and if so, it might possibly not become evident until several generations had been run through the differential varieties.

Some of the races seem to be limited in distribution. This being the case, care should be exercised in the interchange of seed as a precautionary measure against introducing races into areas where they do not already occur.

On the basis of the results obtained, it should be possible to breed soft wheat varieties for the eastern area that would have desirable agronomic characteristics and also be resistant to the 11 races of *Ustilago tritici*.

SUMMARY

Fifty-two collections of *Ustilago tritici* collected from 15 states of the eastern soft wheat region were tested at the Illinois Agricultural Experiment Station, Urbana, Illinois. Eleven physiologic races were pathogenically distinct and have been assigned numbers from 1 to 11.

Race 1 was the most widely distributed and was collected in 7 of the 15 states. Races 4, 8, and 9 were each represented by 1 collection only. Six different races were collected from Texas; five each from North Carolina and Georgia; four from Kentucky; three from each Illinois and South Carolina; two from Indiana; and one each from the other eight states.

The results presented indicate that it should be possible, by hybridization, to combine resistance to loose smut and desirable agronomic qualities in such a way as to obtain a satisfactory variety of soft winter wheat with a high degree of resistance to all of the 11 races of loose smut.

U. S. DEPARTMENT OF AGRICULTURE

AND

ILLINOIS AGRICULTURAL EXPERIMENT STATION,

URBANA, ILLINOIS.

LITERATURE CITED

1. ATKINS, I. M., E. D. HANSING, and WAYNE M. BEVER. Reaction of varieties and strains of winter wheat to loose smut. Jour. Amer. Soc. Agron. 39: 363–377. 1947.
2. BONNETT, O. T., and W. M. BEVER. Head-hill method of planting head selections of small grains. Jour. Amer. Soc. Agron. 39: 442–445. 1947.
3. CALDWELL, RALPH M., and L. E. COMPTON. Cross inoculations with loose smut of wheat. (Abstr.) Phytopath. 30: 3. 1940.
4. GREVEL, F. K. Untersuchungen über das Vorhandensein biologischer Rassen des Flugbrandes des Weizens (*Ustilago tritici*). Phytopath. Zeitschr. 2: 209–234. 1930. (Abstr. in Rev. Appl. Mycol. 9: 708. 1930.)
5. HANNA, W. F. Physiologic forms of loose smut of wheat. Canad. Jour. Res. (C) 15: 141–153. 1937.
6. ————, and W. POPP. Physiologic forms of loose smut of wheat. (Abstr.) Phytopath. 22: 11. 1932.
7. MOORE, M. B. Physiologic races of *Ustilago tritici*. (Abstr.) Phytopath. 26: 103. 1936.
8. ————. Physiologic races of *Ustilago tritici*. (Abstr.) Phytopath. 32: 13. 1942.
9. PIEKENBROCK, P. Untersuchungen über das Verhalten des *Ustilago tritici* an Sorten und Kreuzungen. Kühn-Arch. 15: 411–456. 1927. (Abstr. in Rev. Appl. Mycol. 7: 435. 1928.)
10. POEHLMAN, J. M. A simple method of inoculating barley with loose smut. Phytopath. 35: 640–644. 1945.
11. RADULESCU, E. Untersuchungen über die physiologische Specialisierung bei Flugbrand des Weizens *Ustilago tritici* (Pers.) Jens. Phytopath. Zeitschr. 8: 253–258. 1935. (Abstr. in Rev. Appl. Mycol. 14: 620. 1935.)

CONTROL OF CABBAGE DOWNY MILDEW WITH BENZENE VAPOR[1]

H . H . FOSTER AND J . A . PINCKARD

INTRODUCTION

Commercial cabbage (*Brassica oleracea* var. *capitata* L.) production in Mississippi is centered in Copiah and parts of adjoining counties. The common practice is to sow cabbage seed in coldframes during October and early November, then transplant to the field during late January and February.

Cabbage downy mildew (*Peronospora parasitica* (Fries) Tul.) occurs in this commercial area each year during late fall and winter months. Fungus sporulation is first observed on the cotyledons and later, under favorable conditions, on true leaves. Oospores have been shown to form in the cotyledons (9) and are a possible source of initial infection. Downy mildew or "rust" is the most serious cabbage seedbed disease in Mississippi. In certain seasons this disease becomes epidemic and induces severe losses.

The purpose of this paper is to report experimentation concerned with the development of practical control measures for the control of cabbage downy mildew through the use of benzene vapor. The investigations reported in this paper cover a period of three years, the fall and winter plant-bed seasons of 1943–46. All experiments were conducted at the Mississippi Truck Crops Branch Experiment Station, located at Crystal Springs, Mississippi.

LITERATURE REVIEW

The vapors of certain volatile organic materials were used in investigations on downy mildew of tobacco in 1934 by McLean *et al.* (10). Effective results with benzene vapors were reported by Angell *et al.* (1) from Australia in 1935. Subsequent investigation by both Australian and American workers demonstrated the effectiveness of several organic materials on tobacco mildew under both field and laboratory conditions. A review of the major portion of this work is reported by Wolf (12).

Preliminary work with benzene vapors on the control of cabbage downy mildew in Mississippi (7) indicated that both the survival and size of cabbage plants were increased by nightly application of benzene at the rate of 25 cc. per square yard. Pinckard (11) and Foster (6) have described the practical methods of application and results obtained with benzene.

Symptoms of cabbage downy mildew under southern conditions have previously been described (3, 4). The mode of infection of cabbage seedlings was reported (9); also environmental factors affecting the development

of cabbage downy mildew (5).   A separate report dealing with experimental studies on spray materials for the control of cabbage downy mildew under Mississippi conditions has been prepared.

EXPERIMENTAL PROCEDURE

All experiments were conducted in outside seedbeds or coldframes.   Preliminary experiments to determine rates of benzene, number of applications per week, and type of covers were usually conducted in small frame beds, two square yards in area.   One 15-gram cotton ball was used to vaporize benzene for each 2 square yards of bed space.   Treatments resulting in satisfactory control in the small beds were repeated in larger farm beds.

The seed of several cabbage varieties were used in these experiments.   In each experiment a uniform, weighted amount of seed was sown in all seedbed units of equal size.   No commercial cabbage varieties tested were found to be resistant to the disease.

Washed, unbleached muslin covers of 48 × 44 thread count were used in these experiments.   This type of cover was the same as or similar to coldframe covers used by many cabbage growers.   In some experiments, for comparison, covers with 64 × 64 thread count were also used.   All covers of treated beds, unless otherwise indicated, were saturated with water after the benzene treatments were started.

Small balls of cotton, wrapped in thin muslin and dipped into benzene were suspended by cord from the wooden cover supports.   The benzene treatments were made during the late afternoon and beds remained covered during the night.   Covers were usually removed after approximately 14 to 16 hours of treatment.

Differential data were obtained by recording total number of plants for one square foot, length of seedlings, weight of plants per one square foot, and weight of 100 plants pulled at random.   Weight of plants was based on plant tops, plants being cut at the soil line.   A disease rating scale of zero to four was used in recording disease development.

1943-44 EXPERIMENTS

Experiments were set up to determine the rate and number of benzene applications required to control cabbage downy mildew.

Small-Bed Experiments

Eight small beds (2-square-yard units) were sown to Round Dutch cabbage seed on November 11, 1943.   Beds were covered with 48 × 44 muslin cloth.   All beds were inoculated with a spore suspension on November 23 and the first benzene application was made approximately 36 hours later.   Covers of all treated beds were thoroughly soaked with water in order to better retain the benzene vapors.

Five different treatments were used as shown in table 1.   The first benzene treatment was applied on December 28, 1943.   Beds receiving treat-

ments No. 3 and No. 4 received a total of 35 benzene applications. Beds receiving treatment No. 2 received 12 benzene applications, while beds receiving treatment No. 1 received a total of 13 benzene applications. Final data taken on December 29 and 30, 1943, including explanation of disease rating, are given in table 1.

TABLE 1.—*Summary of benzene treatments (1st series) of cabbage seedbeds in the fall of 1943*

Treatment No.	Treatment	No. of beds treated	Av. no. of plants per ¼ square foot	Av. length of seedlings[a]	Av. disease rating[b]
				Inches	
1	50 cc. per sq. yd. on 3 consecutive nights, first application 36 hours after inoculation	2	102	3.4	1–2
2	Same as treatment No. 1 except that first application was delayed until sporulation appeared	2	79	2.8	3–4
3	12½ cc. per sq. yd. each night, first application 36 hours after inoculation	1	91	4.2	2–3
4	25 cc. per sq. yd. each night, first application 36 hours after inoculation	1	128	4.9	1
5	Control, no benzene treatment	2	79	2.8	3–4

[a] Based on measurements of 20 plants.
[b] Disease rating.
0 = No infection observed.
1 = Infection present. Slight necrosis and usually slight scattered sporulation.
2 = General, abundant sporulation usually present on cotyledons and/or true leaves, with or without death of cotyledons. Usually no stunting. Occasional plants may be killed.
3 = General and abundant sporulation present or previously noted. Cotyledons mostly dropped. Infection and usually sporulation occurring on one or more true leaves. Stunting of plants, definite kill of some plants.
4 = Disease symptoms similar, or more severe, than for rating 3. Death of a large number of plants. (Extremely severe condition approaching optimum for rapid disease development.)

Mildew development was encouraged by watering beds preceding benzene treatments whenever soil and plants appeared dry. The first fungus sporulation was observed on December 3, 1943, ten days following inoculation. The minimum temperature from December 3 to 28, 1943, beneath the bed cover, varied from 26° to 61° F. The maximum temperature, during the same period, varied from 36° to 66° F.

The results of this experiment, summarized in table 1, show that treatment No. 4, or nightly benzene applications at the rate of 25 cc. per sq. yd., gave the best control and indicated that, under the conditions of this experiment, the degree of control was satisfactory. Only one additional treatment,

No. 1, gave fair control. This treatment, 50 cc. of benzene per sq. yd. for three consecutive nights, was promising for control but under more severe conditions might not sufficiently limit disease development. Treatment No. 2 was definitely unsatisfactory, showing that applications of 50 cc. per sq. yd. on three consecutive nights, starting applications following sporulation, did not control the disease. This fact brings out a striking difference in the effectiveness of benzene control of cabbage and tobacco downy mildew, the latter disease having been satisfactorily controlled with benzene following sporulation. Treatment No. 3, or nightly applications of 12½ cc. per sq. yd., with the first application 36 hours following inoculation, failed to give satisfactory control. The benzene usually vaporized during the early part of the night leaving insufficient vapor to inhibit sporulation during the early morning hours. Some plants were observed to have been killed from mildew in beds receiving treatments No. 2 and No. 5.

As shown in table 1, treatment No. 4 gave an average of 128 plants per ½ square foot, an average seedling length of 4.9 inches, and an average disease rating of 1. Treatment No. 1 gave an average of 102 plants, 3.4 inches in length, and an average disease rating of 1 to 2. Treatment No. 5, or untreated control, gave an average of 79 plants, 2.8 inches in length, and an average disease rating of 3 to 4.

Farm-Bed Experiments

In addition to experiments conducted in small 2-sq.-yd. beds, information pertaining to benzene control of cabbage downy mildew was obtained from experiments conducted in three large farm beds, each approximately 70 square yards. Bed No. 2 and No. 3 were sown on October 10, 1943. Bed No. 2 contained several different cabbage varieties while bed No. 3 was sown to Detroit Resistant. Bed No. 4 was sown on November 11, 1943, and contained several cabbage varieties. Small untreated control beds were sown to Round Dutch cabbage seed on each of the above mentioned dates.

All beds were covered with muslin cloth of 48×44 thread count. Covers of treated beds were thoroughly wet following benzene treatments. Sporulation, from natural infection, developed in each bed in advance of benzene treatments.

In beds No. 2 and No. 4 benzene was applied at the rate of 50 cc. per sq. yd. In bed No. 3 benzene was applied at the rate of 25 cc. per sq. yd.

Benzene applications, for beds No. 2 and No. 3, were applied as mildew sporulation indicated the necessity of maintaining practical control, usually 3 successive treatments per week. In bed No. 4 three successive treatments per week were applied regularly, following sporulation.

The first sporulation, in bed No. 4, was observed on December 9, 1943, and the first benzene treatment was applied on December 10. On January 12, 1944, the 21st and final benzene treatment was applied. During December considerable disease developed on cabbage plants in bed No. 4, especially during nights when benzene treatments were not applied, and there was definite stunting and death of some plants.

Bright sunny weather generally prevailed during November and early December and was conducive of only mild disease development. On December 1, 1943, bed No. 2 received the 10th and final benzene treatment; and bed No. 3 received the 13th and final treatment. At this date cabbage plants had developed the 4th and 5th true leaf. Only slight scattered sporulation was present in either treated or untreated beds and severe mildew infection later in the season was not anticipated. Later in December following the termination of the experiment and during a period of cold, rainy weather, beds No. 2 and No. 3 were necessarily covered for several days. During this period mildew sporulation advanced from the lower true leaves to the upper younger leaves. In some places soft rot (as a secondary infection) followed downy mildew. As a result of the two diseases many cabbage plants were killed. This late attack suggested the desirability of continuing benzene treatments approximately to transplanting time.

Results shown in table 1, as well as observational data on large farm beds No. 2, 3, and 4, strongly suggest that benzene applied on three successive nights and at the rate of either 25 cc. or 50 cc. per sq. yd. will not satisfactorily control cabbage downy mildew. These results also suggest that benzene may be less effective fungicidally in the control of cabbage downy mildew than in the control of tobacco downy mildew. Good control of tobacco downy mildew has been obtained, following sporulation, by treating with benzene on 2 to 3 successive nights per week.

1944–45 EXPERIMENTS

Small-Bed Experiments

During the second season of experimental work on the control of cabbage downy mildew, experiments were outlined to further investigate control measures in relation to rate and number of benzene applications. In addition, thread count of bed covers in relation to effective control was considered.

Series 1. Eight different treatments were used as shown in table 2. The first series of small beds were sown with Copenhagen Market cabbage seed on October 19, 1944. Seedbeds were watered in advance of seeding and again following seeding to insure better germination and a more uniform emergence. All 12 beds were inoculated on October 30, 1944, with a spore suspension of the downy mildew fungus. The first benzene treatments were applied approximately 36 hours following inoculation. Mildew development was encouraged by watering beds ahead of benzene treatments, whenever the soil surface appeared dry. All treated beds, with the exception of No. 5, received a total of 52 treatments. The bed receiving treatment No. 5 was given 38 benzene treatments. The final benzene treatment was applied on December 21, 1944. Final data were taken twelve days later, on January 2, 1945, as shown in table 2.

Even with regular watering of beds the mildew attack was not sufficiently severe to kill a high percentage of plants in the untreated control beds.

Plant stand was not particularly uniform as indicated in table 2, and final data failed to give an accurate picture of earlier mildew development. Disease development taken at two different dates for this first series showed marked differences between better treatments and the untreated control.

The main conclusion that can be drawn from this experiment is that the

TABLE 2.—*Summary of benzene treatments (1st series) of cabbage seedbeds in the fall of 1944*

Treatment No.	Treatment	No. of beds treated	Av. no. of plants per 1 sq. ft. (large plants)	Av. wt. of plants per 1 sq. ft. (above soil line)	Av. disease rating[a] Nov. 22, 1944
				Grams	
1	50 cc. per sq. yd. each night under 64 × 64 cover, dry	1	82	285	1
2	50 cc. per sq. yd. each night under 64 × 64 cover, wet	1	99	241	1
3	50 cc. per sq. yd. each night under 48 × 44 cover, dry	1	90	246	2
4	50 cc. per sq. yd. each night under 48 × 44 cover, wet	1	75	280	1
5	50 cc. per sq. yd. on 5 successive nights, skip 2 nights, then repeat treatments, under 48 × 44 cover, wet	1	66	220	1–1.5
6	25 cc. per sq. yd. each night under 48 × 44 cover, wet	2	75	249	2
7	12½ cc. per sq. yd. each night under 48 × 44 cover, wet	2	80	222	2.5
8	No benzene treatment, cover dry each night, 48 × 44 cover	2	85	156	3
8–A	No benzene treatment, cover dry each night, 64 × 64 cover	1	61	126	3

[a] Disease rating. See table 1, footnote b.

higher rates of benzene, 50 cc. per sq. yd., and under both grades of covers induced better control than the lower rates of benzene.

Series 2. Earlier experiments indicated that nightly applications of benzene at the rate of both 50 cc. and 25 cc. per sq. yd. gave fair control. Consequently both rates were again compared under two types of covers, both wet and dry. In this experiment all benzene treatments were applied on 5 successive nights per week.

Ten small (2-square-yard) beds were sown to the Golden Acre variety on November 16, 1944. All beds were inoculated with a spore suspension

on November 28 and the first benzene applications were made November 29, 1944. Six different treatments were used (Table 3), two beds for each treatment. A total of 47 benzene applications was made for each treatment, two treatments during December having been omitted. The final benzene treatment was applied on January 26, 1945, and final data were taken 19 days later on February 14, 1945.

Data given in table 3 show that downy mildew reduced the stand of cabbage plants in untreated control beds. The average weight of plants (above soil line) per square foot was markedly less for plants from untreated beds; also the weight of 100 plants from untreated beds was strikingly lower than the weight of an equal number from treated frames. The best control resulted from treatment No. 1, or 50 cc. per sq. yd. on 5 successive nights under 64 × 64 dry cover, and No. 3, or 50 cc. per sq. yd. on 5 successive nights under
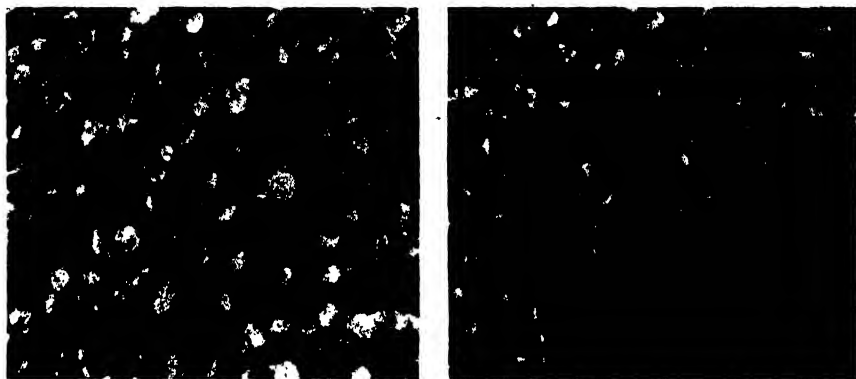
Fig. 1. Plants from Series 2 (2-sq.-yd. beds) 1944–45. A. Treated with benzene at 50 cc. per sq. yd. on 5 successive nights per week under a wet cover of 48 × 44 thread count. B. Untreated control under a wet cover of 48 × 44 thread count. Note sparse stand and stunted plants in untreated control. Approximately same magnification. Photographs taken January 4, 1945, by E. L. Moore.

48 × 44 wet cover. However, differences in stand count and weights between treatments, as shown in table 3, were not markedly high for any one treatment.

Beds receiving treatments No. 1 and No. 3 produced approximately the same average number of plants per square foot, 169 and 171 respectively. Treatments No. 2 (50 cc. per sq. yd. on 5 successive nights under 48 × 44 dry cover) and No. 4 (25 cc. per sq. yd. on 5 successive nights under 48 × 44 wet cover) were less effective in controlling mildew than either treatment No. 1 or No. 3, but gave higher stand counts. Treatment No. 3 is probably the safest treatment of the group and with respect to average weight of plants per square foot was better than other treatments, indicating more vigorous plant growth. The difference in appearance between plants receiving treatments No. 3 and No. 5 (untreated control) is illustrated in figure 1, A and B. When comparison is made of the average weight of 100 plants, data show that with No. 2, one of the poorer treatments, the value is definitely high.

The other benzene treatments, comparing average weight of 100 plants, are relatively close. One possible explanation for these variable results might be the irregular stand within the beds. Although the same weight of seed was sown in each bed, plants were not uniformly and equally spaced. The principal conclusions that should be drawn from this experiment are: (1) All benzene treatments used, under the conditions of this experiment, resulted in both increase of stand and weight over the untreated controls. (2) Treat-

TABLE 3.—*Benzene control of cabbage downy mildew (series 2) in 1944–45*

Treatment No.	Treatment	Av. no. of plants per 1 sq. ft. (large plants)	Av. wt. of plants per 1 sq. ft. (above soil line)	Av. wt. of 100 plants pulled at random (above soil line)	Av. disease rating[a] Jan. 13, 1945
			Grams	*Grams*	
1	50 cc. per sq. yd. 5 successive nights 64 × 64 cover, dry	169	292	226	1.5
2	50 cc. per sq. yd. 5 successive nights 48 × 44 cover, dry	195	270	257	2–2.5
3	50 cc. per sq. yd. 5 successive nights 48 × 44 cover, wet	171	342	233	1.5
4	25 cc. per sq. yd. 5 successive nights 48 × 44 cover, wet	173	289	222	2–2.5
5	No benzene treatment, 48 × 44 cover, dry	155	171	135	3–3.5
6	No benzene treatment, 64 × 64 cover, dry	138	197	158	3–3.5

[a] Disease rating. See table 1, footnote b.

ments No. 1 and No. 3, based on record of disease development, were superior to other benzene treatments tested in this experiment.

Experiments with Large Farm Beds

The object of these experiments was to gain additional information regarding benzene control in cabbage beds similar to those used by commercial truck farmers.

Experiment 1. Four large farm beds were used in these experiments, each bed approximately 66 square yards. On September 25, 1944, the beds were treated with chloropicrin at the rate of approximately 10 lb. per 66 sq. yd. Beds Nos. 4 and 5 were sown on October 17 and beds Nos. 6 and 7 on October 18; all four beds were sown to the variety Copenhagen Market, one lb. seed per bed. Muslin of 48 × 44 thread count was used to cover beds No. 4 and No. 5 while 64 × 64 muslin was used to cover beds No. 6 and No. 7. These four beds were spot-inoculated with a spore suspension on October 30 and again on November 4. Beds No. 4 and No. 7 were used as untreated

controls while beds No. 5 and No. 6 were benzene treated at the rate of 50
cc. per sq. yd.   The first benzene treatment was applied October 31, ap-

FIG. 2.   Comparison of benzene treated and untreated plants.   A. Representative
large plants from farm bed No. 5 (benzene treated) and farm bed No. 4 (untreated).
B. Representative small plants from farm bed No. 5 (benzene treated) and farm bed
No. 4 (untreated).   C. Section from benzene treated farm bed No. 5.   D. Section from
untreated farm bed No. 4.   Approximately same magnification.   A and B taken on
January 4, 1945; C and D taken on November 27, 1944 by E. L. Moore.

proximately 36 hours following inoculation.   A total of 38 benzene applica-
tions were made for beds No. 5 and No. 6.   The final benzene treatment was

TABLE 4.—*Relation of cabbage seedbed cover to benzene control of downy mildew in farm seedbeds, 1944–45*

Bed No.	Treatment	Av. no. of plants per 1 sq. ft.	Av. wt. of plants per 1 sq. ft. (above soil line)
			Grams
6	50 cc. per sq. yd., 64 × 64 cover, wet	73	340
7	Untreated control, 64 × 64 cover, dry	66	220
5	50 cc. per sq. yd., 48 × 44 cover, wet	112	392
4	Untreated control, 48 × 44 cover, dry	81	194

applied December 21, 1944.   Final data were taken eight to eleven days later.   Results of these experiments are shown in tables 4 and 5.

Data shown in table 4 are from beds No. 4, 5, 6, and 7.   These data indicate an increase in number of plants per square foot in benzene treated bed No. 5 over the untreated control beds.   The weight of plants per sq. ft. was in favor of benzene treatments, 340 and 392 grams compared with 220 and 194 grams.   Differences between treated and untreated plants from farm beds No. 4 and No. 5 are shown in figure 2.

Table 5 compares plant stand and weight per square foot in relation to plant size, small, medium, and large, from beds No. 6 and No. 7.   The number of plants per square foot was again variable but the average was in favor of the treated bed, 98 compared with 83 from the untreated control.   The average weight of plants per square foot was markedly higher for the benzene treated bed No. 6, 227 grams compared with 157 for the untreated control.   These results suggest that with downy mildew control larger and stockier plants developed.   These data again suggest that plant stand was more or less variable and taken alone was not entirely reliable in indicating

TABLE 5.—*Plant size in relation to benzene control beneath 64 × 64 muslin*

Seedbed No.	Treatment	Plant size	Plants per 1 sq. ft.	Av. plants per 1 sq. ft.	Wt. of plants per 1 sq. ft.	Av. wt. of plants per 1 sq. ft.
			Number	*Number*	*Grams*	*Grams*
6	Benzene, 50 cc. per sq. yd., 64 × 64 cover, wet	Large	98		324	
6	Do	Medium	89		217	
6	Do	Small	106	98	138	227
7	No benzene, 64 × 64 cover, dry	Large	71		230	
7	Do	Medium	106		150	
7	Do	Small	72	83	92	157

the degree of downy mildew control. Differences in plant size from ben-
zene treated bed No. 5 and untreated bed No. 4 are illustrated in figure 3.

During this experiment many cabbage plants receiving benzene treatment
(50 cc. per sq. yd.) beneath a 64 × 64 cover appeared abnormal, apparently
because of benzene injury. Late in the season, during December, 1944,
many plants showed marked chlorosis on one or more leaves. Plants ap-
peared somewhat stunted, with shortened internodes, and with a general flat-
tened appearance. The difference in appearance between cabbage plants in
bed No. 5 and bed No. 6 was striking, as shown in figure 3. Similar symp-
toms, apparently from benzene injury, were observed in small (2-square-
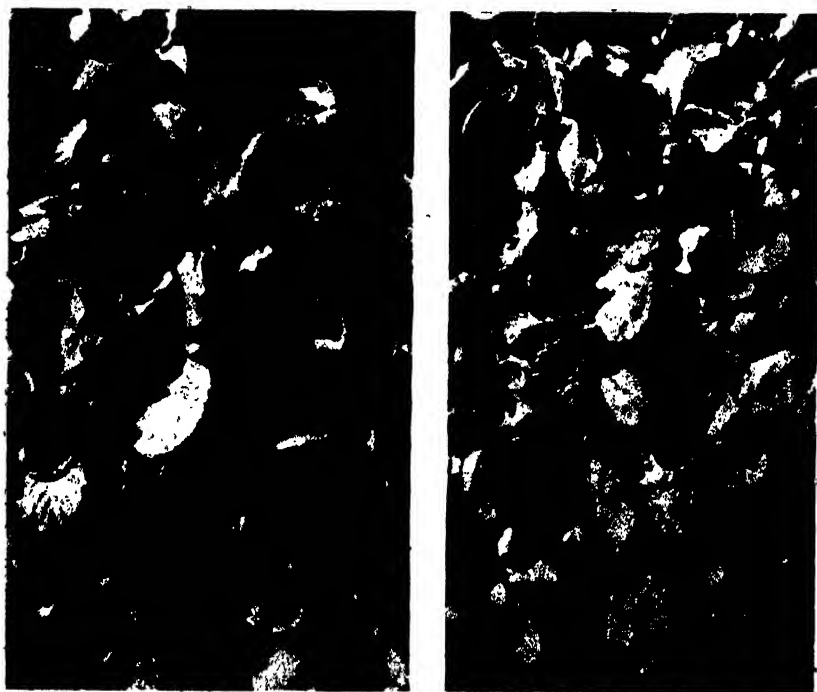
FIG. 3. A. Section from farm bed No. 5 treated with benzene at 50 cc. per sq. yd.
on 5 successive nights per week under a wet cover of 48 × 44 thread count. Note vigorous
healthy plants. B. Section from farm bed No. 6 treated with benzene at 50 cc. per
sq. yd. on 5 successive nights per week under a wet cover of 64 × 64 thread count. Note
flattened distorted plants as a result of benzene injury. Approximately same magnifica-
tion. Photographs taken January 4, 1945, by E. L. Moore.

yard) beds covered with 64 × 64 muslin and receiving 50 cc. of benzene per
sq. yd. These small beds were in the 2nd series of 1944-45.

Experiment 2. On November 17, 1944, two large farm beds, approxi-
mately 66 square yards, were sown to the Golden Acre variety. Both beds
were covered with 48 × 44 muslin. Downy mildew spore inoculation was
made on November 28, 1944. Bed No. 8 received benzene treatments, 25 cc.
per sq. yd., on 5 successive nights per week while bed No. 9 was used as the
untreated control. The first benzene application was made 36 hours after

inoculation.   A total of 42 benzene treatments were applied to bed No. 8, three regular treatments being omitted during December.   The final benzene application was made on January 30, 1945.   Final data were taken 16 days later on February 15, 1945 (Table 6).

Notes pertaining to disease development were taken during the course of the experiment.   On December 19, 1944, very little fresh sporulation was observed in either the treated bed No. 8 or the control bed No. 9.   On December 30, 1944, following a period of cloudy rainy weather (high humidity) abundant fresh sporulation was observed in the control bed No. 9 with only a trace to slight sporulation in the treated bed No. 8.   Sporulation was observed to be more pronounced in low areas of bed No. 8 where drainage was poor and a higher humidity had maintained.   Again on January 13 fresh sporulation was observed in control bed No. 9, with moderate to severe

TABLE 6.—*Benzene treatment (25 cc. per square yard) in relation to plant stand and weight*

Bed No.	Benzene treatment	Av. plants per 1 sq. ft. (large plants)	Av. wt. of plants per 1 sq. ft. (above soil line)	Av. wt. of 100 plants pulled at random (above soil line)
			Grams	Grams
	25 cc. per sq. yd. 5 successive nights per week under 48 × 44 cover, wet	116	232	251
	Untreated control	94	169	219

necrosis on true leaves, slight stunting, and a few plants killed from mildew. In the treated bed No. 8 general moderate necrotic flecking was apparent on nearly all true leaves but with only an occasional plant killed from mildew.   In general, plants appeared stronger and in better condition in bed No. 8 than in bed No. 9.   However, the benzene treatment of 25 cc. per sq. yd. appeared to be barely holding the downy mildew disease from becoming serious.

In table 6 final data are given based on results from beds No. 8 and No. 9. Results are in favor of the benzene treated bed, indicating that during the late fall and early winter, when conditions are frequently favorable for mildew development, benzene treatments at the lower rate of 25 cc. per sq. yd. may, to some extent, check the downy mildew disease.

1945–46 EXPERIMENTS

During the third seedbed season experimental work was continued in relation to rate of benzene to type of coldframe cover required for adequate mildew control.

Early Round Dutch seed were sown on October 31, 1945, at a uniform rate to each of the small (2-square-yard) beds.   Spore inoculation was made

on November 11 and the first benzene treatment was applied on November 12, 1945. The first sporulation was observed on November 18, 1945. The final benzene treatment was applied January 11, 1946. A total of 42 benzene treatments was applied, three regular treatments being omitted during December. Final data were taken on January 29, 1946.

The benzene treatments compared in this experiment, also selected data including explanation of disease rating, are shown in table 7. During previous experiments the first treatment listed (50 cc. per sq. yd. under a 48 × 44 wet cover) has been found consistently to be the most satisfactory treat-

TABLE 7.—*Comparison of different quantities of benzene and different types of cover in the control of downy mildew on cabbage*

Amount of benzene for 1 sq. yd.; condition and type of cover[a]	Plants in 1 sq. ft. of seedbed	Wt. of plant tops in 1 sq. ft. of seedbed	Wt. of 100 plant tops (large plants pulled at random)	Disease rating[b]
	Number	*Grams*	*Grams*	
50 cc., under a 48 × 44 (thread count) wet cover	105	245	546	1–2
50 cc., under a 64 × 64 (thread count) dry cover	80	133	323	2–3
50 cc., under a 48 × 44 (thread count) dry cover	85	158	468	2
35 cc., under a 64 × 64 (thread count) dry cover	96	165	427	2–3
35 cc., under a 48 × 44 (thread count) wet cover	116	262	557	2
25 cc., under a 48 × 44 (thread count) wet cover	84	226	596	2
25 cc., under a 64 × 64 (thread count) dry cover	99	267	726	1.5
No benzene treatment 48 × 44 (thread count) dry cover	85	149	378	2–3

[a] All treatments were applied on 5 successive nights per week in advance of sporulation.

[b] Disease rating. See table 1, footnote b.

ment under severe mildew conditions. The second treatment (50 cc. per sq. yd. under a 64 × 64 dry cover) was found to induce severe benzene injury. The third treatment (50 cc. per sq. yd. under a 48 × 44 dry cover), as in previous experiments, failed to satisfactorily control sporulation during the early seedbed stage. Both the fourth and fifth treatments (35 cc. per sq. yd. under 64 × 64 dry cover, and 35 cc. per sq. yd. under a 48 × 44 wet cover, respectively) were considered unsatisfactory. The fourth treatment, with the 64 × 64 cover, induced benzene injury; roots of many cabbage plants had decay and weight of plants was relatively low. The fifth treatment failed to satisfactorily limit mildew sporulation during the seedling stage. The sixth treatment (25 cc. per sq. yd. under a 48 × 44 wet cover) also failed to control mildew sporulation. The seventh treatment (25 cc. per sq. yd.

under a 64 × 64 dry cover) appeared promising. Cabbage plants grown under this treatment were especially large and vigorous. Although the stand count per square foot was not particularly high, the weights, both per square foot and per 100 plants, were the highest for any treatment. The untreated control was markedly low for stand count and weights. With only one treatment, No. 3, were there fewer plants and lower weight of plant tops per square foot.

This experiment included the first trial using the 25 cc. rate under a 64 × 64 (thread count) dry cover. Sporulation was satisfactorily controlled in this trial and plants were of excellent size and condition. Although promising, additional experiments, including replicated trials, are essential before this treatment can be recommended to cabbage growers.

COST OF BENZENE CONTROL

Cost of control measures must be considered before practical recommendations can be made. Basing cost of benzene on experimental farm bed treatments for 1944–45, benzene at the rate of 50 cc. per sq. yd. would not exceed 50 cents per thousand plants. If the rate of benzene during the latter half of the seedbed season was reduced to 25 cc. per sq. yd. the cost of benzene should not exceed 30 cents per thousand plants. Since plants grown out of the State and trucked into Mississippi frequently sell at $2 to $4 per thousand, the cost of benzene would not be prohibitive.

DISCUSSION

Environmental factors are of primary importance in relation to the development of cabbage downy mildew. In comparing hygro-thermograph records with observations on sporulation and disease development, high humidity has always been of major importance. Sporulation has been observed to occur within a wide temperature range, from approximately 35° to 80° F., providing high humidity approaching a saturated atmosphere occurred during the night. These observations are in close agreement with experimental data presented by other investigators (3, 5).

Experiments conducted during the 1943–44 seedbed season showed that nightly applications of benzene at 25 cc. per sq. yd. resulted in markedly better plants when compared with untreated cabbage beds. Observational data indicated benzene treatments should be continued to within a few weeks of field transplanting.

During the 1944–45 seedbed season 50 cc. of benzene per square yard on 5 successive nights under a 48 × 44 wet cover gave relatively good control. The average disease rating with this treatment was always low and average weight of plants per one square foot was usually high. Under artificially severe mildew conditions this treatment kept downy mildew sporulation under good control.

During the 1945–46 seedbed season benzene treatments at the rate of 50 cc. per square yard on five successive nights per week under a 48 × 44 wet

cover continued to give satisfactory control. Several additional benzene rates were tested under both 48 × 44 and 64 × 64 covers. One treatment, 25 cc. per sq. yd. under a 64 × 64 dry cover, appeared promising. Plants in this treatment were especially large and vigorous, also practically free from downy mildew.

Benzene treatments at the rate of 25 cc. per square yard under a 48 × 44 wet cover frequently failed to satisfactorily control mildew sporulation during the early seedling stage. However, after cabbage plants developed the third true leaf this lower benzene rate did hold mildew sporulation in check.

Benzene applications at the rate of 50 cc. per square yard, under a wet cover of 64 × 64 muslin, induced injury on cabbage plants which became apparent during the latter part of the plant bed season. These results suggest that benzene may be more highly toxic to the cabbage plant than to the tobacco plant. Wolf *et al.* (12) stated that "even when amounts of benzol lethal to the pathogen are applied it becomes very difficult to injure tobacco seedlings under field conditions since the limits of toxicity of benzol to the parasite and to the host are widely separated." Clayton *et al.* (2) stated that the "immediate plant injury from benzol treatment can only result from extreme neglect, as the margin of safety is very great."

Evidence presented by Wolf *et al.* (12) and Clayton *et al.* (2) show that benzene satisfactorily controlled tobacco downy mildew following the appearance of active sporulation. Clayton *et al.* (2) indicated that with covers of 40 and 46 thread count a fair degree of control was obtained and control was markedly improved by increasing the thread count. Benzene experiments with cabbage mildew started following abundant general sporulation and under relatively favorable mildew conditions have failed to satisfactorily control mildew infection.

Three years of experimentation in the control of cabbage downy mildew justify the following conclusions: Benzene at the rate of 50 cc. per square yard should be applied on five successive nights per week under a 48 × 44 wet muslin with applications starting in advance of sporulation. Following development of the 3rd or 4th true leaf benzene applications could safely be reduced to 25 cc. per sq. yd. during the remainder of the treatments. To insure adequate control, benzene treatments should be continued until two to three weeks preceding transplanting to the field. Lower air temperatures during the latter part of the seedbed season make it feasible to reduce the benzene rate. Both benzene vaporization and mildew sporulation are markedly reduced when nightly temperatures, under the covers, reach a minimum of 35° F.

SUMMARY

1. Observational data indicate that humidity is of primary importance in sporulation of the downy mildew fungus.

2. Results from repeated experiments indicate that benzene is effective at the rate of 50 cc. per square yard when applied on five successive nights

per week under a wet muslin cover of 48 × 44 thread count, with applications starting in advance of sporulation and continuing to within a few weeks of field transplanting.

3. Following development of the 3rd or 4th true leaf benzene applications could be reduced to 25 cc. per square yard during the remainder of the treatments.

4. Benzene at 25 cc. per square yard on five successive nights per week under a 64 × 64 dry cover appeared promising.

5. Data were primarily recorded by taking number and weight of plants per square foot, weight of 100 large plants pulled at random, and disease rating based on a scale from zero to four.

6. Cost of benzene treatments was estimated at approximately 30 cents per thousand plants.

MISSISSIPPI AGRICULTURAL EXPERIMENT STATION,
    STATE COLLEGE, MISSISSIPPI.

LITERATURE CITED

1. ANGELL, H. R., A. V. HILL, and J. M. ALLAN. Downy mildew (blue mould) of tobacco: its control by benzol and toluol vapours in covered seed beds. Jour. Council Sci. and Indus. Res. [Australia] 8: 203–214. 1935.
2. CLAYTON, EDWARD E., JOHN G. GAINES, KENDALL J. SHAW, THOMAS E. SMITH, HAROLD H. FOSTER, W. M. LUNN, and THOMAS W. GRAHAM. Gas treatment for the control of blue mold disease of tobacco. U. S. Dept. Agr. Tech. Bull. No. 799. 1942.
3. EDDINS, A. H. Control downy mildew of cabbage with Spergon and Fermate. Fla. Agr. Exp. Sta. Press Bull. 589. 1943.
4. ————————. Protecting cabbage plant beds from downy mildew with Spergon. Proc. Fla. Hort. Soc. 1944.
5. FELTON, MATHIAS W., and J. C. WALKER. Environal factors affecting downy mildew of cabbage. Jour. Agr. Res. [U.S.] 72: 69–81. 1946.
6. FOSTER, H. H. Control of downy mildew or "rust" in cabbage seedbeds. Miss. Agr. Exp. Sta. Circ. 127. 1946.
7. ————————, and J. A. PINCKARD. Control of cabbage mildew by means of benzene vapor. (Abstr.) Phytopath. 34: 1000. 1944.
8. LEBEAU, F. J. Systemic invasion of cabbage seedlings by the downy mildew fungus. Jour. Agr. Res. [U.S.] 71: 453–463. 1945.
9. ————————, and J. A. PINCKARD. Oospore production in cabbage seedlings by Peronospora parasitica. (Abstr.) Phytopath. 32: 648. 1942.
10. MCLEAN, RUTH, FREDERICK A. WOLF, F. R. DARKIS, and P. M. GROSS. Control of downy mildew of tobacco by vapors of benzol and of other organic substances. Phytopath. 27: 982–991. 1937.
11. PINCKARD, J. A. Control cabbage mildew. The Food Packer 26: 63–64. 1945.
12. WOLF, FREDERICK A. Status of investigations of tobacco downy mildew. Phytopath. 29: 194–200. 1939.
13. WOLF, F. A., J. A. PINCKARD, F. R. DARKIS, RUTH MCLEAN, and P. M. GROSS. Field studies on concentration of benzol vapors as used to control downy mildew of tobacco. Phytopath. 29: 103–120. 1939.

SEED TRANSMISSION OF RHIZOCTONIA SOLANI IN RELATION TO CONTROL OF SEEDLING DAMPING-OFF

KENNETH F. BAKER

The possibility that *Rhizoctonia solani* Kühn, a fungus that commonly causes damping-off of seedlings, may be seed borne is disregarded or denied in most plant pathology textbooks and general bulletins (*e.g.*, 3, 5, 16, 22, 23, 26), although there are several reports of such transmission.

Apparently the first record of seed infection by *Rhizoctonia solani* was that of Hedgecock in 1904 (14) on beans. Mycelia and sclerotia were present in the seed coats, having penetrated through pods in contact with the soil. Fulton (11) later showed that such fungi caused damping-off of seedling beans. Its occurrence in bean seed has subsequently been verified (10). Garden peas were shown by Crosier to be infected in 1931–32 and later (7, 8, 10); hairy vetch (9), peanut (10, 12), and subterranean clover seed (6) also have been found to carry *Rhizoctonia*. These records are all on field crops, and it is to be expected that *Rhizoctonia* transmission in such seeds is difficult to detect and regarded as unimportant; it has been observed largely in seed-testing procedures.

This viewpoint has been carried over into the damping-off problem, delaying progress in disease prevention. *Rhizoctonia* is held to be a soil organism, and control procedures are aimed at its elimination or suppression in the soil. In commercial practice, spot infections of damping-off have been attributed to faulty soil treatment or to recontamination, whereas it is now clear that some of them result from infected seed. For plants normally started in flats or beds of treated soil this fact has marked significance.

It is the purpose of this paper to report additional evidence of seed transmission of *Rhizoctonia*, its significance, and methods of prevention, in extension of a previous abstract (2).

SEED TRANSMISSION IN VARIOUS CROPS

Pepper (Capsicum frutescens L.).—During the period, 1936–45, there were grown annually in southern California more than 5700 acres of chili and bell peppers and pimientos. The crop is largely started with transplants from outdoor seedbeds and greenhouse flats. The production of these seedlings, mostly during the cool months of December to March, is recognized by nurserymen as difficult and precarious, and heavy losses have been accepted as inevitable. Farmers have contracted with as many as 6 nurserymen to insure the required number of seedlings. These losses are primarily due to damping-off caused by *Rhizoctonia solani*. Similar difficulties are encountered elsewhere (4, 15, 31).

Soil pasteurization reduced losses from damping-off in commercial nurseries, but spot infections continued to appear in many flats of seedlings.

Tests with the same seed lots were conducted in the University greenhouses. The seed was sown in soil carefully pasteurized in the flats, and was held in a greenhouse protected from contamination. Again *Rhizoctonia* damping-off developed. Some of the seed was placed on sterile black peat in Petri dishes in the laboratory and after a week typical *Rhizoctonia* mycelium was observed growing out from the seeds and from bits of plant debris which accompanied them.

Further studies showed that most, but not all, seed lots carried this fungus, and not uncommonly had 0.3 per cent infected seed. It was later found that seed from individual fruits may have a much higher percentage of infection. For example, seed from a rotting fruit was treated with sodium hypochlorite (0.5 per cent available chlorine) and the seed coats carefully removed. The contents were planted on agar and all developed *Rhizoctonia*, which indicated internal infection of every seed.

Since much of the seed used locally was grown in Orange and San Diego counties, California, the field conditions which gave rise to such infection were investigated. It was found that some of the seed fields had been planted several successive years to peppers. The plants were very vigorous and were bearing so many large fruits that branches frequently were split or bent down by the weight, the fruit continuing to develop on the ground. Heavy applications of mineral fertilizer were customarily used. Large quantities of water were applied by ditch irrigation, and, because of the dense interlocking top growth, the soil surface was almost continuously wet. Under these conditions it was not surprising to find abundant *Rhizoctonia* soil rot of the fruit, as described for pepper (31), tomato (24, 25), and beans (11, 14, 18). Since peppers grown for seed remain on the plant until they start to wither, the losses from rotted fruit are frequently severe. From the standpoint of seed transmission, however, it is partially decayed fruit which is important.

The ripe fruit is picked and run through a chopper to reduce it to small bits. The pulp is placed in large barrels for several days to decompose, and then run through a long sluice box in which the seed settles to the bottom. The seed is dried on screens and later cleaned by machinery to remove most of the extraneous matter. This whole procedure gives ample opportunity for the *Rhizoctonia* mycelium to spread and invade the seeds. It is not surprising that sclerotia of *Sclerotinia sclerotiorum* (Lib.) Schroet. were sometimes found mixed with the seed.

Histological studies were made to determine the location of infection. Bits of seed were embedded in paraffin, and sections 8–12 μ thick were cut. These were stained with safranin in 50 per cent alcohol and counterstained with fast green in 95 per cent alcohol. It was found that infection of the seed may be present at any of the following points:

1. On the surface and in cells of the outer layer of the testa. The mycelium grows over the surface of the seed and may form small sclerotia there (Fig. 1, B, G). Occasionally it penetrates into the lumen of, but probably not through, the lignified cells of the outer layer of the testa.
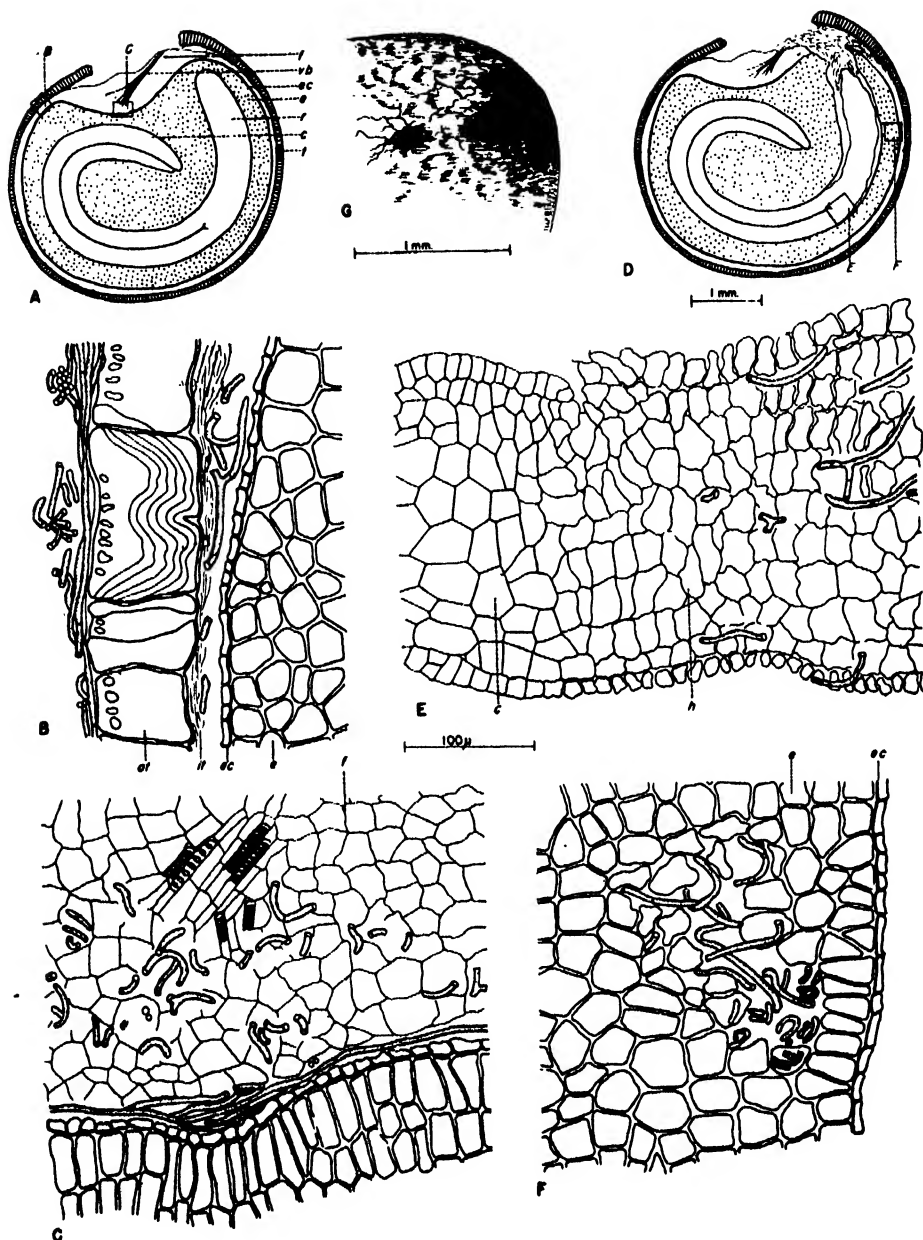
FIG. 1. Camera lucida drawings of bell pepper seed infected by *Rhizoctonia solani*. A–C. Seed with infection of funiculus and testa. B. Mycelium on exterior and in inner layer of testa. C. Mycelium in remnant of funiculus. D–F. Seed with infection of embryo and endosperm. E. Mycelium in hypocotyl. F. Mycelium in endosperm. G. Surface of seed coat, showing mycelium and small sclerotia. c = cotyledon; e = endosperm; cc = endosperm cuticle; f = remnant of funiculus; h = hypocotyl; it = inner layer of testa; ot = outer layer of testa; r = radicle; t = testa; vb = vascular bundle of funiculus. Drawn by K. C. Baker.

2. In crushed cells of the inner layer of the testa (Fig. 1, B). Mycelium enters this area through the funiculus and the opening in the seed coat (Fig. 2, A), and may spread there to surround completely the endosperm cuticle. Examination of dried pepper seed under 72 × magnification will reveal the avenue of such penetration.

3. In the remnant of the funiculus (Fig. 1, C). The pathogen grows into the placenta from the pericarp in contact with the soil, and from the placenta extends into the funiculus. If the mycelium is stopped at this point by the endosperm cuticle (Fig. 1, C), it is still carried with the seed,
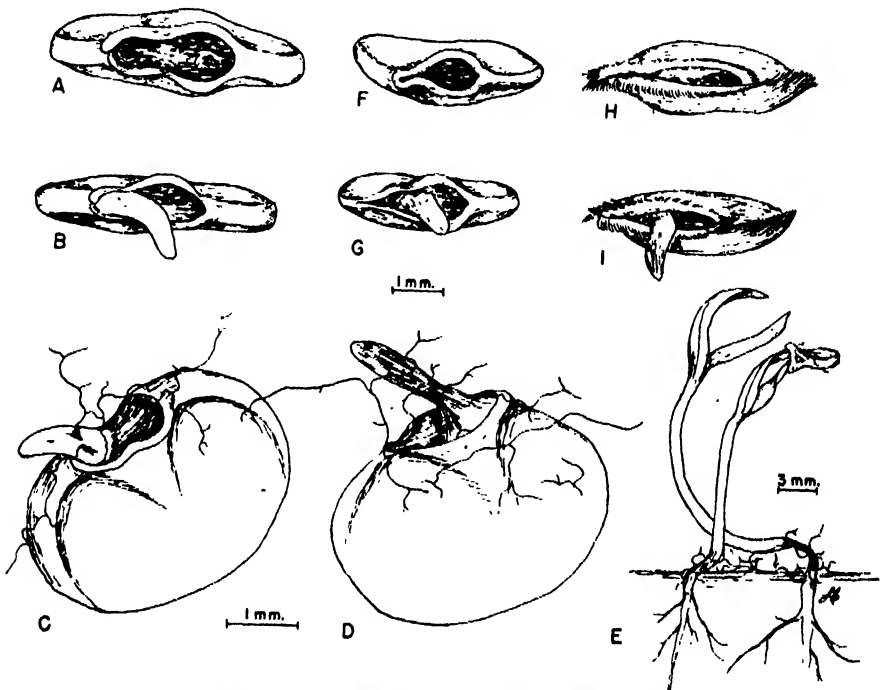
FIG. 2. External seed structure showing opening in testa and dried funiculus through which radicle emerges in germination. A–D. Pepper seeds in various stages of germination and *Rhizoctonia* pre-emergence damping-off. E. Post-emergence damping-off of pepper seedlings. F–G. Eggplant seed germination. H–I. Tomato seed germination. Drawn by L. H. Clark.

and the emerging radicle must push through this infected tissue at germination (Fig. 2, B, C, D). Infection may occur later from seed coats fallen to the ground (Fig. 2, E).

4. In the embryo and/or endosperm (Fig. 1, E, F). Penetration of these structures may occur (a) through the funiculus before it is sealed off by the delayed cutinization of the outer layer of the endosperm, or (b) through a rupture of the endosperm cuticle possibly by growth pressures. In the first type the seed probably would decay, and might be removed during the commercial cleaning process. In type (b) the crack appears in the cuticle of the thin endosperm tissue over the radicle apex, and the radicle and endo-

sperm are invaded. In this circumstance the seed may be dried before there is enough decay to prevent later germination, or rot may proceed so far that no germination occurs; in either case the fungus is carried to the seed-bed where it will spread to adjacent seedlings.

In addition, pentration of the developing ovule or young seed prior to lignification of the testa may occur at any point on its surface and result in complete decay.   ·l.

Although the testa does not completely cover the seed, even at maturity (Fig. 2, A), a cutinized layer forms around the endosperm during develop-ment of the seed.[1] This probably explains why infections are so common in the remnants of the funiculus and inner layers of the seed coat, but less so in endosperm and embryo. It was found, by inoculating fruit at different stages of maturity, that seed transmission is possible even after the seeds have matured.

The fungus appears to digest cells of the embryo or endosperm, at the most, only slightly in advance of the mycelium (Fig. 1, E, F), as found by others (28, 29). The mycelium is both inter- and intra-cellular.

Because of the resistance of *Rhizoctonia* mycelium to drying (13, 26, 29), survival in seeds is to be expected. In the present studies, seed was re-moved from various pepper fruits infected naturally or artificially, washed, dried, and held in seed packets in the laboratory. Seeds harvested in November, 1943, still developed colonies of *R. solani* when planted on sterile black peat 43 months later in May, 1947, although the mycelium in many of the seeds failed to grow.

Tomato (Lycopersicon esculentum Mill.).—Tomato seedlings sustain relatively less damping-off than peppers in southern California, and no evidence of *Rhizoctonia solani* in commercial seed has been obtained. Soil rot of the fruit (24, 25) occurs commonly in seed fields, but the method of seed cleaning is less favorable to spread and growth of the fungus than is the case for pepper. It is probable also that the lower susceptibility of tomato than pepper seedlings plays some part in this situation.

Seed cleaned from inoculated fruit in November, 1944, still developed a few colonies of *Rhizoctonia* on sterile black peat 31 months later in May, 1947. Histological studies revealed mycelium in the remnants of the funiculus and the inner layer of the testa, much as in pepper. There is a much smaller opening in the seed coat of tomato (Fig. 2, H, I) than in pepper, and this may contribute to the lesser importance of *Rhizoctonia* transmission in tomato.

Eggplant (Solanum melongena L.).—Eggplant seedlings are started much as are peppers, and frequently sustain heavy losses from *Rhizoctonia* damping-off. Examination of fruit in the field showed some with *Rhizoc-tonia* soil rot (25) similar to that of pepper, furnishing a possible explana-tion of infected seed. Fruit inoculated with pepper isolates of *Rhizoctonia*

[1] Baker, K. C. Seed anatomy and ontogeny of Capsicum frutescens L. Unpublished manuscript.

decayed and gave infected seed. Commercial seed of eggplants indexed as for pepper gave clear indications of carrying *Rhizoctonia*.

The opening in the seed coat is similar to that of pepper (Fig. 2; F, G) and it is probable that mycelium also is carried in remnants of the funiculus of these seeds.

Zinnia (*Zinnia elegans* Jacq.).—Zinnias generally are sowed in place, but r so are started in flats for nursery sale. Seedlings do not usually sustain losses from *Rhizoctonia* damping-off. It was, therefore, of interest that *Rhizoctonia* should be found in routine testing of zinnia seed.

Commercial zinnia seed lots grown in 3 areas of California were planted in flats of sterile soil in the greenhouse to index for the presence of *Alternaria zinniae* Pape. A number of the lots consistently sustained severe losses from *Rhizoctonia* damping-off in repeated plantings. This was surprising because zinnia flower and seed heads do not normally come into contact with the soil during development.

There are two possible explanations of this phenomenon: (1) In some fields or varieties, plants might be broken or matted down so that flower heads would be in contact with infested soil. It would be unlikely that many such heads would be gathered when hand picking is practiced, but they would not be excluded when the whole plants are put through a threshing machine. (2) Hand-picked flower heads are piled to dry on canvas spread on the ground. Whole plants are sometimes piled on the ground prior to threshing. The bottom layers of either type of pile remain moist and this, plus capillary soil moisture, supplies conditions favorable for the growth of *Rhizoctonia* and infection of the seeds.

SIGNIFICANCE OF SEED TRANSMISSION OF RHIZOCTONIA

The fact that *Rhizoctonia* is seed borne on more kinds of seeds and with greater frequency than is commonly supposed is significant in several ways besides dissemination of the fungus from one area to another (14). Because of the general distribution of *R. solani* in soil, this dissemination has been considered inconsequential, particularly on field crops (8).

Because of the existence of a degree of physiological specialization in *Rhizoctonia solani*, even as it causes damping-off (literature reviewed in 17, 28, 29), dissemination of the fungus with seed assumes more importance. Reciprocal inoculation tests with various isolates from rotting tomato and pepper fruits from commercial fields showed that some infected both tomato and pepper fruits readily, whereas others attacked only one or the other. The concomitant seed carriage provides a means for the continued association of a virulent fruit-rotting *Rhizoctonia* with the appropriate host.

Crops usually started in pasteurized or treated soil probably are most affected by seed transmission of *Rhizoctonia*. It is generally recognized that a pathogen introduced into soil free from competing organisms is able to spread faster and cause more damage than it would in the presence of a normal soil flora (19, 20), although there are exceptions (27). The gross

effect is to make some crops of this type exceedingly difficult to grow unless both soil and seeds are treated.

Since *Rhizoctonia* does not form spores under seedbed conditions, airborne contamination is rare. Bits of mycelium in soil or plant parts may be spattered about in watering, or carried to some extent on tools or flats. Recontamination in these ways can, however, be avoided by careful cultural practices.

CONTROL OR PREVENTION OF SEED TRANSMISSION

Seed treatment.—Because the fungus is carried internally it seemed improbable that chemical treatment would be effective. Since preliminary tests with New Improved Ceresan, Semesan, mercuric chloride, and red copper oxide on infected pepper seed were not promising, hot-water treatment was tried. This proved so effective that no further work was done with chemical treatments.

A hot-water treatment at 51.7° C. (125° F.) for 30 minutes was found to eliminate *Rhizoctonia* seed transmission in chili and bell peppers with slight reduction of germination (greatest reduction with fresh seed was 13.4 per cent). A temperature of 53.9° C. (129° F.) was found to reduce germination so much that it is not recommended. Newton (21) found that cultures of *R. solani* on agar were killed at 50° C. only if exposed for one hour, and that sclerotia on potato tubers were killed by 60° C., but not by 55° C., in one hour. The discrepancy of these data with our results remains unclarified.

Large-scale treatments were begun in 1943, and during 4 seasons many lots, aggregating perhaps a ton of seed, have been treated by us and by nurserymen. In no instance has there been reported any injurious effect and it is concluded, therefore, that the treatment is entirely safe.

In one representative test using 4,000 treated and 4,000 untreated California Wonder Bell pepper seeds, there were 54.6 per cent fewer seedlings suitable for transplanting in the series grown 8 weeks in raw soil free of *Rhizoctonia,* as a result of seed transmission. In the series with untreated seed planted in pasteurized soil there was a 69.5 per cent reduction from those sowed with treated seed. In a commercial nursery 2 to 2½ times as many plants were obtained per seed flat from treated as from untreated seed.

Large lots of seed may be treated in boxes with screen sides. These boxes, about half filled, are plunged into a large tank containing 200–250 gallons of water held by a steam pipe to 51.7° C.; a water pump circulates the water and maintains uniform temperature. No presoak or preheating dip is needed. During the 30-minute exposure the boxes are turned over several times to facilitate uniform heating. At the end of the treatment the boxes are removed, drained, and cooled quickly by flooding with tap water from a hose. The seed is then drained and spread out in thin layers on screens to dry. This may be done outdoors in warm weather, in a heated room with fans, or in a dehydrator, the only requirement being that the seed be dried in 12–20 hours without overheating.

Small lots of seed may be handled in half-filled cheesecloth bags. These are treated as above in a sink or tub of about 30 gallons capacity, temperature being most easily controlled by allowing hot water (60° C., from a water heater) to trickle in so as to hold the temperature at 51.7° C. The bags may be cooled quickly by submerging in cold water or by holding them under the tap.

Only one other recommendation of hot-water treatment of pepper seed has been found (30), using 51.7° C. for 25 minutes, followed by Cuprocide 2½ oz. per 15 lbs., for bacterial spot (*Xanthomonas vesicatoria* (Doidge) Dowson). Hot-water seed treatment was tried (15) for control of pepper diseases in Georgia; its injuriousness probably was due to the 6-hour presoak and high temperature (60° C. for 10–30 minutes) employed. Because of the safety and effectiveness of proper hot-water treatment, it might well be tried against other seed-borne diseases of pepper.

Zinnia seed has been successfully treated the same as peppers, but germination loss may be excessive if the seed is more than a year old. For example, in a test involving 12,000 untreated and 12,000 treated seeds, one-year-old untreated seed of 7 varieties had an average of 65.6 per cent emergence in pasteurized soil, and treated seed 55.4 per cent (a decrease of 15.6 per cent), 2-year-old seed of 4 varieties averaged respectively for untreated and treated lots, 66.0 and 40.3 per cent (a decrease of 38.9 per cent), and 3-year-old seed of a single variety 37.8 and 8.0 per cent (a decrease of 78.8 per cent). Eggplant seed also has been safely treated in the same way.

Cultural practices.—Practices aimed at keeping fruit off of the ground to prevent *Rhizoctonia* "soil rot" will reduce fruit decay. Staking of tomatoes has been suggested (24), and wires stretched on each side of rows of bell peppers grown for seed should reduce fruit decay and seed transmission of *Rhizoctonia*. Sanitation and crop rotation (11) should be of benefit. Careful hand sorting of such large seeds as peas and beans (11) would be desirable. Fungicidal dusts or sprays applied under the plants as for bottom rot of lettuce (29) might be effective, but have not been tried.

In the case of zinnia seed infected from the soil through the canvas on which it is piled prior to threshing, a fungicidal treatment of the canvas might prevent its penetration by *Rhizoctonia*. Several seed growers are using for this purpose canvas treated with mercury or copper compounds for mildew prevention, and this may also prove a practical control of *Rhizoctonia* infection.

IMPROVED GROWING METHODS MADE POSSIBLE BY CONTROL OF DAMPING-OFF

It is possible to reduce damping-off of seedlings in commercial nurseries to insignificance by pasteurization of the soil, hot-water treatment of seed, and reasonably careful handling. This fact has made possible a reexamination of nursery procedures, with improvements in mechanization and labor-saving methods. This is illustrated by improvements adopted since 1944 by one nursery[2] in the raising of pepper seedlings for farm trade.

The usual procedure in southern California for raising peppers in flats is to sow "seed flats" thickly, place them on greenhouse benches, hold them fairly dry to reduce damping-off, and transplant to other flats (100–110 plants per 18 × 18 inch flat) when the seedlings are about 1 to 2 inches high. Because some of the plants may have incipient infections when transplanted, and the soil may carry the organism, these flats also are held relatively dry to minimize losses. When the plants are about 10–12 inches high the flats are placed outdoors to "harden." Because seedlings are produced in the winter months, temperatures are likely to be low, and therefore favorable to *Rhizoctonia* infection of such a "warm" crop as pepper. The usual period from sowing to "hardening" is 80 days or more.

Freedom from damping-off has made possible the sowing of seed in place by means of a special machine, since the number of plants is not reduced from disease. This saves the labor of transplanting, the seedlings do not sustain the usual 10–14-day setback from root injury, and the hazard of spreading virus by handling during transplanting is eliminated.

Since the pathogens are destroyed, it is possible to maintain the flats at a high moisture level during germination. Immediately after the seed is planted in the flat and covered with sterile sand, it is rather heavily watered, all of these processes being handled by machines at the rate of 150 flats per hour. These flats are then stacked directly on top of each other and moved into a closed room held at fairly constant temperature and high humidity. Under these moist conditions the seeds germinate uniformly and shed the seed coats without binding. By periodical examination, the flats can be removed from the germination room and placed on greenhouse benches just after emergence, but before the seedlings elongate. By this means greater uniformity and percentage of germination are obtained while avoiding the laborious watering required by seed flats. The greenhouse benches are freed during the 7–14-day germination period, and are used only for plants which require light. This same method of germinating seed flats under conditions free of pathogens is now being successfully used by several nurserymen and seedsmen in California and the mid-West. It has been found useful when a single variety is sowed in numerous flats, but is not desirable where several varieties are involved, because of differences in germination rate. It will be recognized that this method is an adaptation of the practice of covering a flat or pot with a glass plate during germination.

When the seedlings are placed in the greenhouse the humidity may be kept high, and the temperature up to 80° F., with the soil at a high moisture and fertility level. While not yet tried, constant-level watering or subirrigation could be used with still greater reduction of labor. About 50 days are required from seeding to "hardening" with this method of handling.

The flats are moved outdoors for 2 to 3 weeks to harden the plants, during which time most of the leaves abscise and the stems become tough and wiry. The plants are pulled from the flats by hand and, after the dirt is shaken from the roots, placed in celery crates for delivery to the field. Experience

indicates that these plants start rapidly in the field, but with the above method plants of almost any degree of hardness can be produced. It is now generally conceded by farmers who have used the plants that root systems of non-transplanted stock are at least as good as those of seedlings pricked out of seed flats.

The total effect is to produce healthier plants more dependably and quickly and with less labor and expense, in a given greenhouse area. Because the plants are free of virus diseases and such organisms as root-knot nematode and *Rhizoctonia, Pythium, Phytophthora,* or *Sclerotinia,* which cause root, stem, and fruit decay, and of *Verticillium* which causes wilt, the hazard of introducing them to uninfested fields is eliminated. Because of the savings effected by these improved methods, it is probable that plants can now dependably be grown in greenhouses as cheaply as they can with uncertainty in outdoor seedbeds.

It cannot be too strongly emphasized that the success of this method depends on using soil and seed free of pathogens, and on rigorous sanitation, and that without these conditions losses actually may be accentuated.

<div align="center">DISCUSSION</div>

The soil-inhabiting nature of *Rhizoctonia solani* has been so emphasized in phytopathological literature that the traditional control procedures against seedling damping-off aim at elimination or suppression of the fungus in the soil. However, it is now established that the fungus is disseminated in the seed of bean, pea, vetch, peanut, clover, pepper, eggplant, tomato, and zinnia. Such transmission may be the means of reinfesting treated soil, and has resulted in heavy losses in nursery production of pepper plants.

Seed infections of these various crops have in common the fact that they originate from contact of the fruit or flower head with the soil. Pea and bean, and probably vetch and clover, seed are infected on the side through the pod wall. This is analogous to the situation reported for *Macrophomina phaseoli* (Maubl.) Ashby on lima bean (1).

In the case of pepper, eggplant, and tomato, the "soil rot" may spread through the fruit and infect the seed from the placenta. The fungus may penetrate the developing ovule, or young seed with unlignified seed coat, at any point on its surface and produce decay. After the seed coat has lignified, the area of penetration apparently is restricted to the opening through which the funiculus passes. As the endosperm cuticle forms, the area of infection is further restricted. The point of union with the funiculus is the last to be cutinized and, until it has been sealed off, constitutes a bridge to penetration of the endosperm. Thus in the development of the seed there are decreasing areas of possible penetration of the endosperm and embryo by pathogens. However, vulnerable spots remain. The mycelium may grow directly from the funiculus into the inner layer of the testa surrounding the endosperm cuticle; it may remain dormant in this layer and in the funiculus until seed germination, when it infects the emerging

radicle.   Growth cracks may develop in the endosperm cuticle, particularly in the region of the tip of the radicle, allowing mycelium to enter the endosperm or embryo.   The pathogen may grow over the surface of the seed at any time, forming resting mycelia and sclerotia there, and even penetrating into, but not through, the external cells.   *Rhizoctonia solani* has been reported (28, 29) to penetrate the host: (a) between or through epidermal cells (potato, cotton, tomato fruit, lettuce, pea); (b) through cuticle (cotton, kale, lettuce); (c) through lenticels (tomato fruit); (d) through stomata (grass, lettuce); (e) through wounds.   Only (a) and (e) seem to be involved in pepper.

In the case of zinnia, there is direct invasion of the seed by fungus growth into the flower heads piled on canvas on the ground.   Bits of infected stem, fruit, or other tissues which may accompany seed have the same practical result as infected seed, and are not uncommon in commercial packets.

In all of the examples studied, it is an academic point whether infected seed is killed before planting, or is infected and killed after germination. In either case the mycelium quickly radiates to other seeds and causes pre- and post-emergence damping-off.   Equally academic is the question as to whether or not the types of infection described for pepper are all internal. However, it would appear that, to maintain a significant distinction between internal and external seed transmission, the latter must be restricted to cases where detached spores, bits of mycelium, etc., are mechanically lodged on the surface of the testa and grow no further until the seed is planted.

SUMMARY

Seed transmission of *Rhizoctonia solani* has been demonstrated as common in living and dead seeds of bell and chili pepper.   The fungus occurs as mycelia or sclerotia on the surface of the seed, or as mycelia in the attached remnants of the funiculus, the inner layers of the seed coat, the endosperm, or the embryo itself, particularly at the tip of the radicle. Infection was found to originate in fruits rotted with *Rhizoctonia* from contact with soil in the field.

Similar seed transmission has been found with eggplant, tomato, and zinnia, and was previously reported for bean, pea, hairy vetch, peanut, and subterranean clover.[3]

Such transmission is significant because: (a) it introduces strains of the fungus to new areas or fields; (b) it contaminates disinfested soil, causing severe seedling loss; (c) it assures the continued association of a virulent strain of the fungus with the appropriate host.

Hot-water treatment of pepper, eggplant, or zinnia seed at 51.7° C. (125° F.) for 30 minutes killed the fungus in and on the seed without significantly reducing germination.

[3] Since this paper was written, reports of two additional examples of seed transmission of *R. solani*, on spinach and Japanese hop, have been found:
Neergaard, P. Aarsberetning fra J. E. Ohlsens Enkes Plantepatologiske Laboratorium (København) 5: 5. 1940; 6: 4. 1941; 7: 6, 14. 1942; 8: 6. 1943; 10: 6. 1945.

By treatment of both soil and seed to kill the fungus, coupled with practical sanitation, new techniques for growing seedlings have been developed which give more dependable, faster production of better plants in a smaller greenhouse area than do the usual methods, with less labor and expense, and with reduced chance for carrying virus, fungus, or nematode diseases to the field.

DIVISION OF PLANT PATHOLOGY,
    UNIVERSITY OF CALIFORNIA,
        LOS ANGELES, CALIFORNIA.

LITERATURE CITED

1. ANDRUS, C. F. Seed transmission of *Macrophomina phaseoli*. Phytopath. 28: 620–634. 1938.
2. BAKER, K. F. Transmission of *Rhizoctonia solani* in pepper seed and its prevention. (Abstr.) Phytopath. 37: 359. 1947.
3. BEWLEY, W. F. Diseases of glasshouse plants. 208 pp. Ernest Benn, Ltd., London. 1923.
4. CASSELL, R. C. Diseases and injuries of greenhouse-grown vegetable plants in New York. U. S. Dept. Agr., Pl. Dis. Reptr. 29: 520–522. 1945.
5. CHESTER, K. S. The nature and prevention of plant diseases. 584 pp. Blakiston Co., Philadelphia. 1942.
6. CHILTON, ST. J. P. Some pathogenic fungi occurring in the seed of red and subterranean clover. Phytopath. 32: 738–739. 1942.
7. CROSIER, W. F. Detection and identification of seed-borne parasites. Assoc. Off. Seed Anal. No. Amer. Proc. 27: 87–92. 1936.
8. ——————. Prevalence and significance of fungous associates of pea seeds. Assoc. Off. Seed Anal. No. Amer. Proc. 28: 101–107. 1936.
9. ——————. Occurrence and longevity of *Ascochyta pisi* in seeds of hairy vetch. Jour. Agr. Res. [U.S.] 59: 683–697. 1939.
10. ——————. Seed-borne microorganisms. N. Y. (Geneva) Agr. Exp. Sta. Ann. Rept. 62: 56–57. 1943.
11. FULTON, H. R. Diseases of pepper and beans. La. Agr. Exp. Sta. Bull. 101. 1908.
12. GARREN, K. H., and B. B. HIGGINS. Fungi associated with runner peanut seeds and their relation to concealed damage. Phytopath. 37: 512–522. 1947.
13. GRATZ, L. O. Wire stem of cabbage. N. Y. (Cornell) Agr. Exp. Sta. Mem. 85. 1925.
14. HEDGECOCK, G. G. A note on Rhizoctonia. Science 19: 268. 1904.
15. HIGGINS, B. B. The diseases of pepper. Ga. Agr. Exp. Sta. Bull. 141. 1923.
16. HORSFALL, J. G. Combating damping-off. N. Y. (Geneva) Agr. Exp. Sta. Bull. 683. 1938.
17. HOUSTON, B. R. Culture types and pathogenicity of isolates of *Corticium solani*. Phytopath. 35: 371–393. 1945.
18. HYRE, R. A. Diseases in eastern Tennessee. U. S. Dept. Agr., Pl. Dis. Reptr. 27: 326–329. 1943.
19. JOHNSON, J. Soil-steaming for disease control. Soil Sci. 61: 83–91. 1946.
20. LUDWIG, R. A., and A. W. HENRY. Studies on the microbiology of recontaminated sterilized soil in relation to its infestation with *Ophiobolus graminis* Sacc. Can. Jour. Res. (C) 21: 343–350. 1943.
21. NEWTON, W. The physiology of Rhizoctonia. I. The thermal death point of vegetative cultures. Sci. Agr. 12: 178–179. 1931.
22. ORTON, C. R. Seed-borne parasites—a bibliography. W. Va. Agr. Exp. Sta. Bull. 245. 1931.
23. OWENS, C. E. Principles of plant pathology. 629 pp. John Wiley & Sons, New York. 1928.
24. RAMSEY, G. B., and A. A. BAILEY. The development of soil rot of tomatoes during transit and marketing. Phytopath. 19: 383–390. 1929.
25. ——————, and G. K. K. LINK. Market diseases of fruits and vegetables: tomatoes, peppers, eggplants. U. S. Dept. Agr. Misc. Publ. 121. 1932.
26. ROTH, L. F., and A. J. RIKER. Life history and distribution of Pythium and Rhizoctonia in relation to damping-off of red pine seedlings. Jour. Agr. Res. [U.S.] 67: 129–148. 1943.

27. SANFORD, G. B. Studies on *Rhizoctonia solani* Kühn. V. Virulence in steam sterilized and natural soil. Can. Jour. Res. (C) **19**: 1–8. 1941.
28. STOREY, I. F. A comparative study of strains of *Rhizoctonia solani* (Kühn) with special reference to their parasitism. Ann. Appl. Biol. **28**: 219–228. 1941.
29. TOWNSEND, G. R. Bottom rot of lettuce. N. Y. (Cornell) Agr. Exp. Sta. Mem. 158. 1934.
30. WALKER, J. C., et al. Vegetable disease control measures recommended for the Upper Mississippi Valley for 1943. Amer. Phytopath. Soc. War Emergency Comm. Mimeo. Rept. 10. 1943. (Also in Mimeo. Rept. 25. 1944.)
31. WEBER, G. H. Diseases of peppers in Florida. Fla. Agr. Exp. Sta. Bull. 244. 1932.

PHYTOPATHOLOGICAL NOTES

Ceratostomella ulmi on Elm Bark Treated with 2,4-dichlorophenoxy-acetic Acid.—Variations in length and diameter of coremial stalks of *Ceratostomella ulmi* (Schwarz) Buisman on elm and in pure cultures have been reported by Clinton,[1] Schwarz,[2] and Wollenweber.[3] The writers have observed such variations in coremia growing in bark beetle galleries in elm and in cultures from bark beetles. The common range in length is 0.25 to 1.5 mm. Wollenweber[3] refers to coremia up to 3 mm. in length and illustrates unusual coremial forms which suggest some similarity to the

Fig. 1. Coremia of *Ceratostomella ulmi* on elm bark treated with 2,4-dichloro-phenoxyacetic acid, photographed after 65 days. Large group flattened by cover dish, and few coremia of normal size. Photograph slightly retouched. 4x.

types reported herein. In 1933 the senior writer observed extremely long coremiumlike and apparently sterile synnemata on potato-dextrose-agar slants. Again in 1935 on potato-sucrose-agar, a culture of *C. ulmi*, growing with secondary fungi, showed very abundant overgrowths of large fertile coremia slightly over 3 mm. in length. In one instance in 1946 an atypical culture on nutrient agar medium produced fasciated groups of coremia up to 3 mm. in length. In 1947 when isolates from different geographic locations in the United States were grown together on sterilized elm twigs, coremia from 3 to 4 mm. in length were produced.

[1] Clinton, G. P., and Florence A. McCormick. Dutch elm disease, *Graphium ulmi.* Conn. Agr. Expt. Sta. Bul. 389: 701–750. 1936.
[2] Schwarz, M. B. Die Zweigdurre und die Gefasskrankheit der Ulme. Kapitel II *in* Das Zweigsterben der Ulmen, Trauerweiden und Pfirsichbaume, eine vergleichend-pathologische Studie. 32 pp. Utrecht. 1922. (Transl. by L. D. Kelsey *in* Bartlett Res. Labs. Bul. 1: 5–25. 1928.)
[3] Wollenweber, H. W. Das Ulmensterben und sein Erreger, *Graphium ulmi,* Schwarz. Nachrichtenbl. f. den Deut. Pflanzenschutzdienst, No. 10. 1927. (Transl. by L. D. Kelsey *in* Bartlett Res. Labs. Bul. 1: 26–31. 1928.)

In September, 1946, while using chemicals in an attempt to retard growth of secondary organisms and to hasten coremia formation of *Ceratostomella*
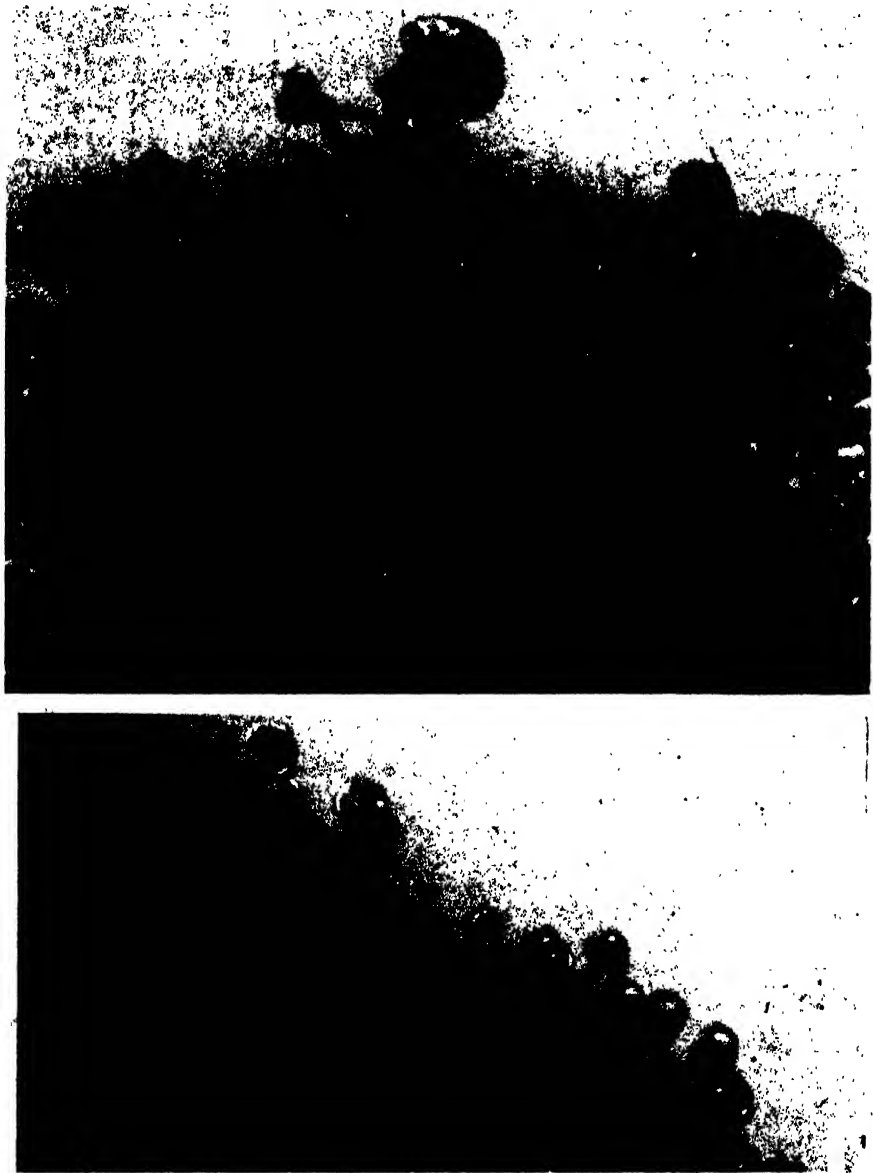
FIG. 2. Coremia of *Ceratostomella ulmi* on elm bark treated with 2,4-dichloro-phenoxyacetic acid, photographed after 65 days: Upper photograph, abnormally large fasciated coremia and synnemata, other large immature forms, and numerous smaller coremia developing from yeast-like spore mass. Lower photograph, coremia of normal size and form. Photographs slightly retouched. 4×

ulmi, several groups of abnormally large coremia were observed. In all instances these abnormal growths were on sterilized elm bark that had been

placed in Petri dishes containing 10 to 15 cc. of a saturated solution of 2,
4-dichlorophenoxyacetic acid or the ammonium salt of this acid in concen-
trations varying from one to six grams per 800 cc. of sterilized water.
Previous tests had indicated that stronger solutions inhibit the growth of
C. ulmi.   After the bark was inoculated with a spore suspension of *C. ulmi*,
the cultures were incubated for 45 days or about three times longer than
usually required for normal coremia production.   The resulting groups of
abnormal coremia varied from 7 to 20 mm. in diameter. These large
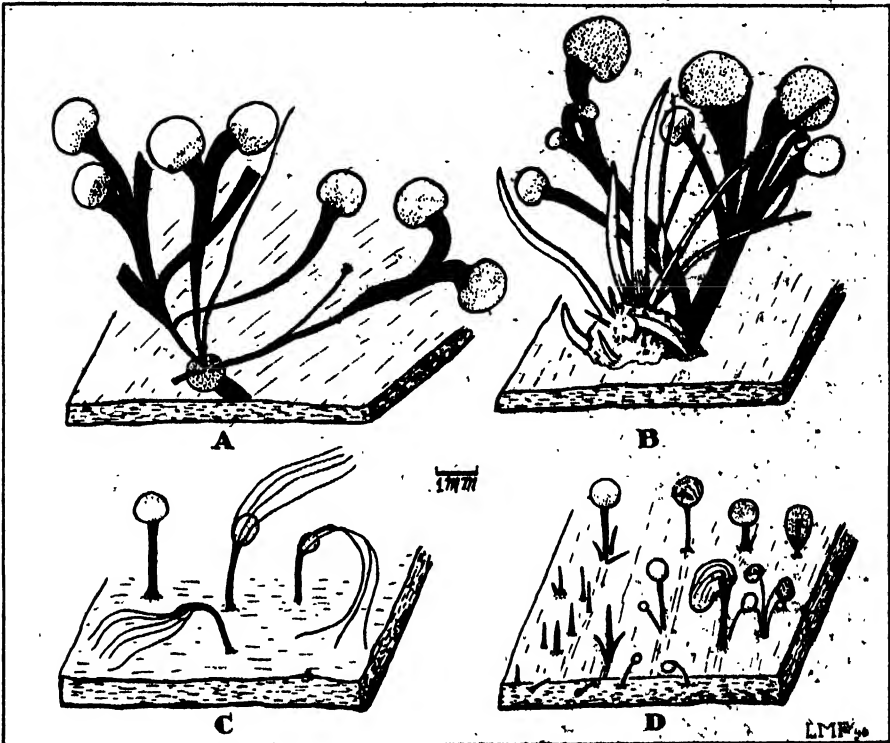
FIG. 3. Diagrams of *Ceratostomella ulmi* coremia on elm bark treated with 2,4-
dichlorophenoxyacetic acid: A, Prostrate, fasciated coremia arising from coremial spore
mass on bark surface; B, Upright group developed from yeast-like spore mass, and im-
mature hyaline stalks as in figure 2, A; C, Primary coremia of normal length with fila-
mentous, whiplike extensions; and D, Comparative size and form of normal mature and
immature coremia on untreated elm bark in moist chamber.

overgrowths appear to have emerged from large masses of yellowish spore
material on the surface of the inner bark. The sizes of the abnormally
large coremial masses may be compared with those of a group of normal
coremia in figure 2.   These growths are illustrated diagrammatically in
figure 3.

In the fasciated groups (Fig. 1) the individual coremia were 8 to 10
mm. in length from the base of the stalk to the top of the coremial head.
The diameter or width of the tubular and flattened stalks ranged from 0.25

to 1 mm. Within the large coremial groups there were long, hair-like synnemata or dark brown filaments more or less than 3 mm. in length (Fig. 2). These hair-like filaments, which extended above the coremial head, seemed to originate from the top of the stalk. Their tips were hyaline and possibly fertile. On each flat, fasciated stalk a coremial head developed into a broadly elliptical mass of spores instead of the usual spherical mass. Subcultures from these abnormally large heads produced apparently normal mycelial colonies at the end of 20 days on potato-sucrose-agar.—LAWRENCE M. FENNER and LESTON R. FATE, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture, East Orange, New Jersey.

Cinchona Root Disease Caused by Phytophthora cinnamomi.[1]—Nurseries for the production of cinchona planting stock for experimental purposes have been maintained at the Estación Experimental Agrícola de Tingo María since early 1943, although it was recognized that the altitude (2,200 ft.) was below the generally accepted optimum for this species. Development of the plants has been somewhat faster than at the regular plantation sites, but, until the past year, diseases other than top die back[2] have not been troublesome. Root disease caused by *Phytophthora* has been absent or unnoticed until the attack herein reported was observed. At the height of the wet season of 1945–46, one-year-old transplants of the Ledger form of *Cinchona officinalis* L. developed chlorosis, progressively followed by loss of all but the terminal leaves on the branches and main stem and within a month by death. The first plants to develop symptoms were in the lower portions of the beds, but by the end of the wet season the disease had involved the entire nursery consisting of about fifteen 100-foot beds. Examination of the root systems of the diseased plants disclosed that the collar region and larger roots were in good condition, but that the smaller rootlets were brown and either dead or diseased.

Phytophthora cinnamomi Rands was isolated from almost all of the rootlets cultured. *P. cinnamomi* has been reported as causing cinchona root rot in British Malaya.[3] However *P. cinnamomi* does not appear to have been heretofore reported as a pathogen on cinchona in the Western Hemisphere.

The disease may be reproduced on healthy seedlings by inoculation only under conditions of high soil moisture. Isolates of *Phytophthora cinnamomi* from cinchona are indistinguishable from isolates from root rot of

[2] Crandall, Bowen S. and William O. Davis. Phytophthora wilt and stem canker of cinchona. Phytopath. 35: 138–140. 1945.

[3] Thompson, A. Notes on plant diseases in 1939. Malayan Agr. Jour. 28: 400–407. 1940.

avocado, *Persea americana* Mill. from the same region, but cross inoculations have not been made to determine if strain differences exist.

This disease should not be confused with the root disease, which is one of the limiting factors in Peruvian cinchona plantations.[4] The *Phytophthora* causing this disease is distinct from *P. cinnamomi* and has been described as a new species, *Phytophthora quininea*.[5]—BOWEN S. CRANDALL, Pathologist, Office of Foreign Agricultural Relations, United States Department of Agriculture and Chief, Department of Plant Pathology and Entomology, Estación Experimental Agrícola de Tingo María.

The Occurrence of Monilinia seaveri on English Morello Cherry.—During a period of 17 years spent investigating fruit diseases in northwest Arkansas the writer frequently has observed the conidial stage of *Monilinia seaveri* (Rehm) Honey attacking the leaves and fruit of mature wild cherry (*Prunus serotina* Ehrh.) trees as well as producing a blight[1] of the seedlings.

In spite of the widespread distribution of the fungus on this wild host, repeated examinations of cultivated plums and cherries in the vicinity of infected wild-cherry trees have failed, except in the two instances described in this note, to reveal infections of cultivated species by this fungus.

On May 8, 1934, one immature cherry on an English Morello (*Prunus cerasus* L.) tree at the University of Arkansas farm north of Fayetteville, Ark., was observed to be infected with a *Monilinia* decidedly different in appearance from the imperfect stage of the common brown-rot fungus, *Monilinia fructicola* (Winter) Honey.

A microscopic examination of the fungus on this single specimen showed conidia and disjunctors identical in appearance with those of *Monilinia seaveri*. The specimen likewise had the characteristic sweetish odor regularly associated with the presence of *M. seaveri* on wild-cherry trees.

No additional specimens were observed in 1934, but on April 29, 1935 the same fungus was found attacking the small, immature fruit on a number of English Morello cherry trees at the University farm. The common brown-rot fungus was also present on other fruit and the two fungi could be distinguished readily in the field. The conidia of *Monilinia fructicola* were present as cushion-shaped masses, while those of *M. seaveri* were closely appressed to the surface of the fruit, as may be seen in figure 1.

No attempts were made to infect wild-cherry leaves with conidia from the English Morello fruit. However, the marked similarity in appearance of the spore masses on the two hosts and the presence of the characteristic sweetish odor on the English Morello fruit, coupled with a microscopic study of spores and disjunctors from both hosts, indicate that the English Morello fruit was attacked by *Monilinia seaveri*.

[4] Crandall, Bowen S., and William C. Davis. Occurrence of Cinchona root rots in the Americas. U. S. Dept. Agr., Plt. Dis. Reptr. 28: 926–929. 1944.

[5] Crandall, Bowen S. A new Phytophthora causing root and collar rot of cinchona in Peru. Mycologia 39: 218–223. 1947.

[1] Dunegan, J. C. A blight of wild cherry seedlings. Phytopath. 30: 89–90. 1940.

Fig. 1. Immature English Morello cherries partially covered with masses of conidia of *Monilinia seaveri*. The two cherries on the left, infected by *M. fructicola*, are included for comparison.

This natural infection of a cultivated host by *Monilinia seaveri* occurred during periods of excessive rainfall, which favored the profuse development of fungus diseases. Similar environmental conditions occurred in northwest Arkansas in 1939 and 1945, but no further cases of the infection of cultivated hosts by *M. seaveri* have been observed.—JOHN C. DUNEGAN, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, Plant Industry Station, Beltsville, Maryland, cooperating with the Arkansas Agricultural Experimental Station.

Thelephora terrestris on Blueberry Plants.—The smothering or strangling fungus *Thelephora terrestris* Fr. (*T. laciniata* Fr.) has been reported as causing injury to forest tree seedlings, especially to conifers. The damage recorded was due to the covering or enveloping of the small seedlings in propagating beds by the fruiting structures of the fungus.

Weir[1] reported the occurrence of the fungus in the United States on *Abies*, *Picea*, *Pinus*, *Pseudotsuga*, and *Thuja* of coniferous forms, and on *Acer*, *Quercus*, and *Ribes*. All these reports of occurrence of *Thelephora terrestris*, except that for *Ribes*, came from western and central States.

In November, 1946, the writer observed the fungus[2] on 1-year-old blueberry (*Vaccinium australe* Small) nursery plants near Toms River, N. J. This is believed to be the first report of *Thelephora terrestris* in association with *Vaccinium*.

The nursery where the fungus was found was planted in inadequately drained sandy virgin soil and contained considerable muck and other unde-

[1] Weir, James R. *Thelephora terrestris, T. fimbriata,* and *T. caryophyllea* on forest tree seedlings. Phytopath. 11: 141–144. 1921.
[2] John A. Stevenson, Div. Mycology and Disease Survey, identified the fungus as *Thelephora terrestris* Fr.

composed plant material. The plants were propagated from cuttings made in 1945 and had grown one year in the nursery rows. The plants were small and well spaced and produced very little shade to favor the growth of the fungus. Although no test was made of the pH of the soil, it undoubtedly was low as blueberries thrive best in acid soils. The fungus was attached to about 2 dozen plants, all confined to an area of about 20 square feet on one side of the nursery and adjacent to uncleared land.

The fungus structures, surrounding the plant stems at and immediately below the ground level, were partly exposed and on account of their large size were easily seen. They varied from 1 to 2 inches in diameter. Figure 1

FIG. 1. *Thelephora terrestris* on a 1-year-old blueberry plant. Toms River, N. J. (× 1.)

is a photograph showing a fungus fruiting body about natural size, on a blueberry plant, after it had been thoroughly cleaned. When the fungus was collected the size of the body was even more impressive, as in addition to the fungus structure there was a considerable amount of soil and plant fragments all tied together in a compact mass by hyphae.

The fungus was loosely attached to the plants and could easily be broken off. There was no evidence of injury done except to some leaves that were in contact with the soil. Those leaves were completely covered with the fungus and a few were dead and had abscised.—J. B. DEMAREE, Plant Industry Station, Beltsville, Maryland.

INDEX FOR VOLUME 37

ERRATA, VOLUME 36

Page 909, line 12, *read* f. muscadinii *for* var. muscadinii

Page 1045, footnote 6, line 2 *read* It would be expected *for* It would not expected

Page 1047, line 28, *read* refers to G. venetum Speg. as used by Scribner (to S. necator) *for* refers to G. venetum Speg.

ERRATA, VOLUME 37

Page 62, line 9, *read* rust-immune *for* rust-immue·

Page 80, line 9, *read* leaf symptoms *for* leafy symptoms

Page 306, table 2 head, *read* shown graphically in figure 3 *for* shown graphically in figure 2

Page 308, table 3 footnote, *read* shown graphically in figure 3 *for* shown graphically in figure 2

Page 318, citation 29, *read* Experientia *for* Experimentia

Page 353, lines 4 and 5, *read* ever showed a trace of the pigment or forms over showed a trace of the pigment of forms

Page 585, figure 2, cut inverted

Page 624, line 15, *add* The writer is indebted to the Carnegie Trust for grant towards the cost of the illustrations.

Page 651, table 2 *read* pod peas *for* pot peas

read Difference required for significance :

5 per cent level.. 27.7

1 per cent level ....   ]

Page 655, line 20, *read* Arasan *for* Arason

Page 847, line 29, *read* which latter symptom is characteristic of tobac mosaic virus *for* such symptoms that are characteristic of tobacco mos virus